Faculty of Medicine

Department of General Practice and Community Medicine

Section for International Health

The most common causes of and risk factors for diarrhea among children less than five years of age admitted to Dong Anh Hospital, Hanoi, Northern Vietnam

Student: Bui Viet Hung

A thesis submitted to University of Oslo as a partial fulfilment for the degree Master of Philosophy in International Community Health

Supervisors:

Gunnar Bjune, Professor, M.D, Ph.D

Department of General Practice and Community Medicine

University of Oslo - Norway

Nguyen Binh Minh, Associate Professor, M.D, Ph.D

Department of Bacteriology

National Institute of Hygiene and Epidemiology (NIHE)

Hanoi - Vietnam.

UNIVERSITY OF OSLO **NIHE**

Oslo, May 2006

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ABBREVIATIONS

AIDS	**:**	Acquired immune deficiency syndrome
APW	**:**	Alkaline pepton water
CDD	**:**	Control of diarrhoeal diseases
CI	**:**	Confidence interval
DALYs	**:**	Disability adjusted life years
EAggEC	**:**	Entero aggregative *Escherichia coli*
E. coli	**:**	*Escherichia coli*
EIA	**:**	Enzyme immuno assay
EIEC	**:**	Entero invasive *Escherichia coli*
EPEC	**:**	Entero pathogenic *Escherichia coli*
ETEC	**:**	Entero toxigenic *Escherichia coli*
GDP	**:**	Gross domestic product
GMP	**:**	Good manufacturing practices
HIV	**:**	Human immunodeficiency virus
HUS	**:**	Haemolytic uraemic syndrome
IMCI	**:**	Integrated management of childhood illness
LDC	**:**	Lysine decarboxylase
MOH	**:**	Ministry of Health
MOR	**:**	Matched odds ratio
NHPs	**:**	National health programs
NIHE	**:**	National Institute of Hygiene and Epidemioly
NOK	**:**	Norwegian kroner

OR	**:**	Odds ratio
ORS	**:**	Oral rehydration salts
ORT	**:**	Oral rehydration therapy
PBS	**:**	Phosphate buffered saline
TCBS	**:**	Thiosulfate citrate bile salt sucrose
UIO	**:**	University of Oslo
UNICEF	**:**	United Nations International Children's Emergency Fund
USAID	**:**	United States Agency for International Development
USD	**:**	United states dollar
WHO	**:**	World Health Organization

ABSTRACT

Background: Acute diarrheal disease among children younger than 5 years old remains a major cause of morbidity and mortality worldwide. Severe infectious diarrhea in children occurs most frequently under circumstances of poor environmental sanitation and hygiene, inadequate water supplies, and poverty. In Vietnam, the control of diarrhoeal disease (CDD), including promotion of breast-feeding, oral rehydration therapy and specific health education is a part of national strategies aiming to improve the quality of life and reduce the burdens caused by diseases. Despite this fact, diarrheal disease is still the second leading cause of infectious morbidity and mortality in children as well as in adults in Vietnam. The local epidemiology of diarrhea in most rural areas of Vietnam has not been researched thoroughly. In addition, most studies in Vietnam have focused on a specific pathogen rather than identifying the most common pathogens of diarrhea among children in rural areas. Better understand the local epidemiology of diarrhoeal disease could be a valuable contribution to the development of public health prevention. We therefore conducted a study in Dong Anh Hospital in order to identify risk factors for diarrhea among children less than five years of age in this area.

Objectives: the study aimed to identify the most common causes of and risk factors for diarrheal disease among children aged less than five years admitted to Dong Anh Hospital, Hanoi.

Method and materials: a hospital-based case-control study was performed. A case was defined as a child less than 5 years of age having three or more loose, liquid, or watery stools or at least one bloody loose stool within the last 24 hours. Accordingly, all cases admitted to Dong Anh Hospital between July and December 2005 which fulfilled the inclusion criteria were recruited into the study. Controls were non-diarrheal patients matched for sex and age. Face-to-face interviews based on the questionnaire were conducted with mothers on the day of admission. Stool samples were collected from all cases immediately after their admission, and were then processed for bacterial, parasitological, and viral studies.

Results: A total of 600 study subjects, including 200 cases and 400 controls, were recruited into the study. Cases were mostly children less than 24 months of age. The number of boys was higher than girls in nearly all age groups.

In multivariate analysis, using conditional logistic regression, some factors remained independently associated with the risk of diarrhea, namely *the child having sibling(s)* (OR=1.9; 95% CI 1.2 - 3.2); *irregular latrine cleaning* (OR=4.4; 95% CI 2.4 - 8.1); *latrine-sharing among more than 5 people* (OR=2.8; 95% CI 1.3 - 6.2); *irregular hand washing by mothers after going to toilet* (OR=4.5; 95% CI 2.1 - 9.5); *no hand-washing by mothers before feeding children* (OR=9.4; 95% CI 2.3 - 37.6); *unsafe storage of food for later use* (OR=3.4; 95% CI 2.0 - 5.7); *irregular kitchen cleaning*(OR=4.3; 95% CI 2.5 - 7.4); and *infrequent cleaning/emptying of storage container before refilling it with fresh water* (OR=7.7; 95% CI 4.4 - 13.5).

Among 200 stool samples collected in the study, we detected 54 cases positive to entero pathogenic *Escherichia coli (EPEC),* 50 cases to *rotavirus* and 8 cases to *Shigella spp.* Co-infecton of *rotavirus-EPEC* was found in 13 cases, and *rotavirus-Shigella* in one case. Infection with *Entamoeba hystolytica* was also detected in 23 cases.

Conclusion: From this study we identified the risk factors of diarrhea to be irregular hand-washing by mothers after going to toilet, no hand-washing by mothers before feeding children, the child having sibling, unsafe storage of food for later use, irregular kitchen cleaning, infrequent cleaning/emptying of storage container before refilling it with fresh water and irregular latrine cleaning, latrine-sharing among more than 5 people. *EPEC*, *Rotavirus* and *Shigella spp*. are found to be common pathogens for diarrhea among children admitted to in Dong Anh Hospital.

From these findings we suggest that encouraging mothers, through education, to wash their hands before feeding their children or after going to toilet should be a priority. Improving hygienic practice in the community through education programmes participated by volunteers, mothers' support groups, health workers, mass media; building kindergartens in all villages; implementing community IMCI (Integrated Management of Childhood Illness); and establishing intersectoral collaboration are the main methods we wish to recommend in order to improve public awareness of diarrhea, eventually aiming to reduce burden caused by diarrhea among children less than five years of age in the district.

Key words: diarrheal disease; risk factors; epidemiology; pathogens; children under five years of age; rural areas; Vietnam.

ACKNOWLEDGEMENTS

I would like to express my dearest thanks to:

- Professor Gunnar Bjune, head of Section of International Health, Department of General Practice and Community Medicine, University of Oslo, Norway, for his great support, encouragement and valuable comments that helped me to attend and complete the Master Degree in International Community Health.
- Associate Professor Nguyen Binh Minh, head of Microbiology Department, NIHE, Hanoi, Vietnam, for her great support and her important and constructive comments on the study.
- Associate Professor Vu Tan Trao, head of Immunology and molecular biology Department, NIHE, Hanoi, Vietnam, for her recommendation to the course and her support during the study.
- Associate Professor Vu Sinh Nam, Vice director of Medical Preventive Department, MOH, for his recommendations to the course.
- Dr Nguyen Van Hoa, head of Microbioly Laboratory, Hanoi Friendship Hospital, for his support to the study.
- Professor Haakon E. Meyer, Department of General Practice and Community Medicine,UIO, for his comments on the study.
- Professor Phung Dac Cam, head of Enteric Pathogens research unit, Microbiology Department, NIHE, Hanoi,Vietnam, for his comments on the study.
- Dr Hein Stigum, Norwegian Institute of Public Health and Dr Magne Thoresen, Department of General Practice and Community Medicine, UIO, for their comments on data analysis of the study.
- My colleagues at Enteric Pathogen Laboratory, Microbiology Department, NIHE, Hanoi, Vietnam for their important help during the fieldwork.
- Directorate and staff in Dong Anh Hospital for their collaboration in the study.
- Mothers and their children for their participation in the study.
- All staffs in Section for International Health, my friends and classmates for their help during the course.

- My parents, my wife and my beloved son, my brother and sister for their love, encouragement and support.

This study was supported by the Norwegian Agency for Development Cooperation (NORAD); Section for International Health, Department of General Practice and Community Medicine, University of Oslo; and National Institute of Hygiene and Epidemiology, Hanoi, Vietnam.

INTRODUCTION

It is over 150 years since John Snow closed the Broad Street pump after a cholera outbreak and thereby initiated the debate on diarrheal disease risk factors and their elimination. Today diarrhea remains a major public health problem. In developing countries, diarrhea is among the leading causes of childhood morbidity and mortality. An estimated one billion episodes and 2.5 million deaths occur each year among children under five years of age. About 80% of deaths due to diarrhea occur in the first two years of life [1, 2]. Many times this number have long-term complications like malnutrition, growth retardation, and immune impairment. Overall, these children experience an average of 3.2 episodes of diarrhea per child per year [2]. Although the majority of diarrheal episodes are not severe and may not require specific intervention, a large number are potentially fatal.[3]

Diarrhea is the most important public health problem connected to water and sanitation and can be both “waterborne” and “water-washed”. In recent decades, a consensus developed that the key factors for the prevention of diarrhea are sanitation, personal hygiene, availability of water and good quality drinking water; and that the quantity of water that people have available for hygiene is of equal or greater importance for the prevention of diarrhea as the bacteriological water quality [4].

In Vietnam, the control of diarrhoeal disease (CDD), including promotion of breast-feeding, oral rehydration therapy and specific health education is a part of national strategies aiming to improve the quality of life and reduce the burdens caused by diseases. Despite this fact, diarrheal disease is still the second leading cause of infectious morbidity and mortality in children as well as in adults in Vietnam.

Risk factors vary with the child’s age, the pathogens involved, and the local environment. To our knowledge, most studies conducted in Vietnam have not analyzed risk factors according to different age groups and local environment. On the other hand, those studies have mostly focused on the molecular epidemiology of specific pathogens, such as *rotavirus, Escherichia coli, Shigella spp*. My study aimed to identify the most common pathogens, and age-specific and local risk factors for diarrheal disease among children aged less than five years admitted to Dong Anh Hospital, Hanoi. Identification of pathogens and risk factors, and then recommendations of simple, immediate, and effective risk-reduction measures would help local health care services to reduce morbidity and mortality due to diarrhea among young children in the area.

CHAPTER 1

LITERATURE REVIEW

1.1. DEFINITION OF DIARRHEA

Almost everyone has become ill of, or will be affected by diarrhea at some point in their lives. Diarrhea can occur as a symptom of many different illnesses, as a side effect of some drugs or may be due to anxiety among other things. Diarrhea results from an imbalance in the absorption and secretion properties of the intestinal tract; if absorption decreases or secretion increases beyond normal, diarrhea results. It can range in severity from an acute, self-limited annoyance to a severe, life-threatening illness.

The definition of diarrhea depends on what is normal for the individual. For some, diarrhea can be as little as one loose stool per day. Others may have three daily bowel movements normally and not be having what they consider diarrhea. According to K. Armon, diarrhoea is defined as a change in bowel habit for the individual child resulting in substantially more frequent and/or looser stools [5].

Although changes in frequency of bowel movements and looseness of stools can vary independently of each other, changes usually occur in both. Clinical features vary greatly depending on the cause, duration, and severity of the diarrhea, on the area of bowel affected, and on the patient's general health.

In children, the strict definition of diarrhea is excessive daily stool volume**,** more than the upper limit of around 10 g/kg/day [6]. It is certainly possible to have diarrhea by this definition with stools that are at least partially formed, or to not have diarrhea even with liquid bowel movements. As a practical matter, it is seldom possible for a physician to determine exactly how many grams per day of stool a child is having. You must therefore use the history to estimate for yourself whether true diarrhea is present. The history would usually provide most of the information you require to classify the diarrhea by type and to consider the diagnostic approach [6].

1.2. THE MAIN CAUSATIVE AGENTS OF DIARRHEA

Though some diarrhoeas are due to errors of metabolism, chemical irritation or organic disturbance, the vast majority are caused by infectious pathogens [7].

Bacterial infections: Diarrhea caused by enteric bacterial infections is very important worldwide, especially in tropical and developing countries, and is a serious problem among older children and adults as well as in infants and young children. The range of causative microorganisms is very large; they include *E. coli*, *Salmonella, Shigella, Campylobacter, Yersinia*, *vibrios,* and *Clostridium difficile* [8].

Viral infections: *Rotavirus* is one of the most common causes of severe diarrhea. Other viruses may be important causes of diarrheal disease in human, including *Norwalk virus*, *Norwalk-like viruses*, *enteric adenoviruses*, *caliciviruses*, and *astroviruses* [8].

Parasites: Parasites can enter the body through food or water and settle in the digestive system. Parasites that cause diarrhea include *Giardia lamblia, Entamoeba histolytica, Cyclospora cayetanensis* and *Cryptosporidium*.

Food intolerances: Some people are unable to digest some component of food, such as lactose - the sugar found in milk, or gluten found in wheat and barley.

Reaction to medicines, some kinds of antibiotics (such as clindamycin, cephalosporins, sulfonamids…), laxatives and antacids.

Intestinal diseases like inflammatory bowel disease or celiac disease.

Functional bowel disorders, such as irritable bowel syndrome, in which the intestines do not work normally.

1.3. TRANSMISSION ROUTES

Infectious diarrhea is acquired by fecal-oral transmission that includes consumption of contaminated food or water, person-to-person contact, or direct contact with fecal matter. With regard to water-borne-diarrhea, transmission patterns occur when in-house water storage facilities or/and water sources are contaminated (corresponding to domestic domain and public domain contamination) [4, 9]. Most of transmission of diarrhea occurs in the domestic domain.[4]

According to Curtis V [10], there are four transmission routes that the major infectious agents use to reach human hosts, namely human-to-human via the environment; human-to-human multiplying in the environment; human-to-animal-to-human via the environment; and animal-to-human via the environment. In situations where faecal contamination of the domestic environment is high, the majority of cases of endemic disease probably occurs either by human-to-human transmission, or from the human-to-human transmission of pathogenic agents which have multiplied in the environment.

1.4. TYPES OF DIARRHEA

Diarrhea may be classified into four general types, based on the mechanism, including osmotic diarrhea, secretory diarrhea, exudative diarrhea, and motility disorder diarrhea [11]. According to WHO [2], Vesikari T and Torun B [3], and Banerjee B, Hazra S and Bandyopadhyay D [12], based on clinical syndromes, diarrhea could be classified into four types, each reflecting a different pathogenesis, including acute watery diarrhea, dysentery, persistent or prolonged diarrhea and chronic diarrhea.

Acute watery diarrhea: this term refers to diarrhea characterized by abrupt onset of frequent, watery, loose stools without visible blood, lasting less than two weeks. Usually, acute watery diarrheal episodes subside within 72 hours of onset. It may be accompanied by flatulence, malaise and abdominal pain. Nausea, vomiting may occur and also fever may be present. The common causes of acute watery diarrhea are viral, bacterial, and parasitic infections. Bacteria also can cause acute food poisoning. The enteric pathogens causing this diarrhea in developing countries are largely the same that are encountered in developed countries, but their proportions are different. In general, bacterial pathogens are more important in countries with poor hygienic conditions. The most important causes of this diarrhea in developing countries are *Rotavirus, Shigellae,* entero toxigenic *E. coli* (ETEC), *Vibrio cholerae, Campylobacter jejuni,* entero pathogenic *E. coli* (EPEC), *Salmonella spp*. and *Cryptosporidium* [3].

The most dangerous complication is dehydration that occurs when there is excessive loss of fluids and minerals (electrolytes) from the body. With vomiting, dehydration becomes more severe. Dehydration is especially dangerous in infants and young children due to rapid body water turnover, high body water content and relatively larger body surface [13]. Patients with mild dehydration may experience only thirst and dry mouth. Moderate to severe dehydration may cause orthostatic hypotension with syncope (fainting upon standing due to a reduced volume of blood, which causes a drop in blood pressure upon standing), a diminished urine output, severe weakness, shock, kidney failure, confusion, acidosis (too much acid in the blood), and coma.

Dysentery may simply be defined as diarrhea containing blood and mucus in feces. The illness also includes abdominal cramps, fever and rectal pain. The most important cause of blood diarrhea is *Shigella*. *Shigella* is a genus of bacteria with four species: *S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei.* In developing countries, the main causative agents of dysentery are *S. flexneri, S. boydii* and *S. dysenteriae,* whereas *S. sonnei* is the main cause in developed countries [14]. *S. dysenteriae* type1 (Sd1) is responsible for epidemic shigellosis. *S. dysenteriae* type1 can result in severe complications including persistent diarrhea, septicemia (blood poisoning), recta1 prolapse and haemolytic-uraemic syndrome (HUS). HUS is a serious condition affecting the kidneys and blood clotting system. *S. flexneri, S. boydii* and *S. sonnei* are usually less dangerous than *S. dysenteriae* type1 and they do not cause large epidemics [15].

Evidences showed that around 10 percent of diarrhoeal episodes in children under five years of age have visible blood in the stool. This 10 percent of episodes causes about 15 percent of diarrhea-associated deaths in this age group [16]. Disease caused by *S. dysenteriae* type1 tends to be more common in infants, and elderly and malnourished people. Mortality is also highest in these groups.

Other pathogens causing endemic dysentery in children include: *Campylobacter jejuni,* invasive strains of *E. coli* (EIEC), non-typhoid *Salmonella* strains and *Entamoeba histolytica* [15]. *Entamoeba histolytica* usually causes less than 2 percent of episodes of bloody diarrhoea in children less than 5 years old [16].

Persistent diarrhea is defined as diarrheal episodes of presumed infectious aetiology that have an unusually long duration and last at least 14 days [3, 13]. About 10 percent of diarrheas in children from developing countries become persistent, especially among those less than three years and more so among infants. The episode may begin acutely either as watery diarrhea or dysentery. This diarrhea causes substantial weight loss in most patients. It may be responsible for about one-third to half of all diarrhea-related deaths. Since persistent diarrhea is a major cause of malnutrition in the developing countries, even the milder, non-fatal episodes contribute to the overall high mortality rates that are frequently associated with malnutrition in these countries.

The pathogenesis of persistent diarrhea is not fully known. Several causes, probably in combination, include: infections with entero aggregative *E. coli* (EAggEC), EPEC and *Cryptosporidium*; intolerance to foods; delayed recovery of intestinal mucosal damage due to protein-energy malnutrition or Vitamin A or zinc deficiency; immunodeficiency (with the exception of Acquired Immune Deficiency Syndrome - AIDS causing chronic diarrhea); and inappropriate use of antibiotics [3].

Chronic diarrhea: This term refers to diarrhea which is recurrent or long lasting due to mainly non-infectious causes. Chronic diarrhea may be caused by gastrointestinal disease, may be secondary to systemic disease, may be psychogenic in nature [3, 11]. Pathophysiologically, chronic diarrhea may be categorized as inflammatory diarrhea (caused by regional enteritis, ulcerative colitis), osmotic or malabsorptive diarrhea (resulted from lactose intolerance, tropical sprue, celiac disease, Whipple's disease, chronic pancreatitis, bile duct obstruction), secretory diarrhea (caused by medications, bowel resection, mucosal disease), dysmotility diarrhea (caused by conditions such as diabetic neuropathy or irritable bowel syndrome) and factitious (self-induced, e.g., from laxative abuse) diarrhea [5, 11].

1.5. RISK FACTORS FOR DIARRHEA

Demographic factors: Many studies have established that the diarrhea prevalence is higher in younger children [13, 17, 18, 19, 20, 21, 22]. The prevalence is highest for children 6-11 months of age, remain at a high level among the one year old children, and decrease in the third and fourth years of life [13, 17, 21, 22]. Higher rate of diarrhea has been observed in boys than girls [13, 19, 21, 23].

Other demographic factors, like mothers' younger age[18, 22], low level of mother's education[13, 17, 18, 24, 25, 26], high number of siblings [17, 27], birth order [28], were significantly associated with more diarrhea occurrence in children less than five.

Socio-economic factors: Some studies have shown that the association between socio-economic factors, such as poor housing, crowded conditions[13, 17, 19, 24], low income [13, 17, 24]; and higher rate of diarrhea was statistically significant.

Water-related factors: As diarrhea is acquired via contaminated water and foods, water-related factors are very important determinants of diarrhea occurrence. Increasing distance from water sources [22, 28], poor storage of drinking water [4, 19, 21, 22] (e.g. obtaining water from storage containers by dipping, no drinking water storage facility), use of unsafe water sources (such as rivers, pools, dams, lakes, streams, wells and other surface water sources)[18, 20, 23, 25, 26, 29, 30], water storage in wide-mouthed containers [9, 30], low per capita water used [25, 26], have been found to be risk factors for more diarrhea occurrence among children less than five..

Sanitation factors: Sanitation obviously plays a key role in reducing diarrhea morbidity. Some sanitation factors, like indiscriminate or improper disposal of children's stool and household garbage [21, 25, 26, 30, 31], no existence of latrine [17, 22, 27, 31] or unhygienic toilet [24, 25], sharing latrine [29], house without sewage system [31], increased the risk for diarrhea in children.

Hygiene practices: Some studies have revealed that children not washing hand before meals or after defecation [22, 29, 32, 33, 34], mothers not washing hands before feeding children or preparing foods [22, 29, 32, 34], children eating with their hands rather than with spoons [31], eating of cold leftovers [23], dirty feeding bottles and utensils [21, 30, 34], unhygienic domestic places (kitchen, living room, yard)[17, 24, 33, 34], unsafe food storage[34], presence of animals inside the house [23, 34], presence of flies inside the house [34], were associated with risk of diarrhea morbidity in children.

Breastfeeding: The literature on feeding practices and risk of diarrhea is extensive. In general, the morbidity of diarrhea is lowest in exclusively breast-fed children; it is higher in partially breast-fed children, and highest in fully-weaned-children [13, 20, 35, 36, 38]. In addition, a particular risk of diarrhea is associated with bottle-feeding [13, 30]. Many studies have shown the strong protective effect of breast feeding. A high concentration of specific antibodies, cells, and other mediators in breast milk reduces the risk of diarrhea following colonization with entero pathogens [13].

Malnutrition**:** the association between diarrhea and malnutrition is so common in low income societies that the concept of a vicious circle is appealing, with diarrhea leading to malnutrition and malnutrition predisposing to diarrhea[13, 39]. Children whose immune systems have been weakened by malnutrition are the most vulnerable to diarrhea. Diarrhea, especially persistent and chronic diarrhea, undermines nutritional status, resulting in malabsorption of nutrients or the inability to use nutrients properly to maintain health. A number of studies have reported higher incidence of diarrhea in malnourished children [13, 39, 40]. A tendency of increased incidence of diarrhea was also found in children with low weight-for-age, or, in particular, in stunted children [23.]

Immunodeficiency: Immunodeficiency is not only a cause of persistent or chronic diarrhea (chronic diarrhea is the major cause of morbidity and death among adults with Human immunodeficiency virus - HIV) [2, 3], but also a risk factor for diarrhea. Due to innate or acquired immunodeficiency, patients are vulnerable to pathogens that cause infectious diseases including diarrhea. Diarrhea is reported in up to 60% of patients with AIDS [41]. One of the many consequences of the HIV/AIDS pandemic may be to halt the impressive decline in childhood diarrheal mortality seen over the past four decades. Diarrheal incidence, duration, severity and mortality are higher in children with HIV/AIDS than in others [2].

Seasonal distribution: Seasonal patterns to childhood diarrhea have been noted in many tropical locations, where there are two definite seasonal peaks: the summer one, associated with bacterial infections, and the winter one, related to viruses [8]. In some studies diarrhea prevalence was found to be higher in the rainy season than in the dry season [8, 42]. During the dry seasons when rainwater and borehole water are less available, disinfecting drinking water from available surface sources may substantially reduce illness [29]. In some studies contamination was more prominent during the rainy season [22, 43, 44].

According to A. Teshima *et al* [45], the number of diarrhea patients in the first peak in April is sensitively correlated to climate elements in pre-monsoon. Climate in pre-monsoon influences the total number of diarrhea patients through the spring peak (April-May) and the climate in August through October influences the autumn peak of patients. Meteorological elements play reverse role on the peak of spring and autumn diarrhea patient. There are also some researches reporting that a distinct increase of diarrhea takes place in the years of El Nino [46, 47, 48].

Consumption of food sold by street vendors: This is also a significant risk factor [29]. Tourists visiting foreign countries with warm climates and poor sanitation can acquire diarrhea by eating contaminated foods such as fruits, vegetables, seafood, raw meat, water, and ice cubes [8].

Eating habits: Eating with the hands; eating raw foods; or drinking unboiled water, may increase the risk of diarrhea.

1.6. THE GLOBAL BURDEN OF DIARHEAL DISEASE IN CHILDREN

Diarrhea is a global problem, but is especially prevalent in developing countries in conditions of poor environmental sanitation, inadequate water supplies, poverty and limited education [49]. According to WHO, approximately one billion cases of diarrhea occur each year worldwide causing a burden that was about 99.2 million DALYs (*disability adjusted life years)* lost. It is well known that diarrheal disease is one of the leading causes of illness and death in young children in developing countries. Diarrhea accounts for 21% of all diseases causing deaths at below five years of age and causes 2.5 million deaths per year, although diarrhea morbidity remains relatively unchanged, about one billion episodes or 3.2 episodes per child-year [2, 49, 50, 51].

1.7. IMPACT OF DIARRHEAL DISEASE ON CHILDREN

The number of deaths caused by diarrhea, 2.5 millions yearly is a large burden. In addition, many time this number have long-term, lasting effects on nutritional status, growth, fitness, cognition, and school performance [2, 25, 49]. Some studies have revealed the impact of diarrhea on growth [8, 13, 52, 53, 54]. It is believed that diarrhea have a significant impact on growth due to reduction in appetite, altered feeding practices and decreased absorption of nutrients [49]. Patwari AK [52] quoted that there was a marked negative relationship between diarrhoea and physical growth and development of a child. Each day of illness due to diarrhoea produces a weight deficit of 20-40 grams. Molbak *et al*[13] found that infants who spent more than 20 % of their time with diarrhea had a weight deficit of approximately 370 grams at follow-up after 1 year of age. There was also an impact on height and that impact varied by age and sex. For example, during infancy, boys who spent from 20% to less than 40% of their time with diarrhea were 5.1 mm shorter than who had no diarrhea, whereas the deficit in girls was negligible. At age of 1-4 years, with the same time spent with diarrhea, the deficit on height was 2.1 mm and 3.0 mm in boys and girls respectively[13]. According to Checkley W. *et al* [53], children ill with diarrhea 10% of the time during the first 24 months were 1.5 cm shorter than children who never had diarrhea. In addition, the adverse effects of diarrhea on height varied by age. Diarrhea during the first 6 months of life resulted in long-term height deficits that were likely to be permanent. In contrast, diarrhea after 6 months of age showed transient effects. Similarly, Molbak [13] and Briend [55] indicated that after 6 months of age, the effect of diarrhea on growth was transient due to catch-up growth.

According to M. Gracey [8], the greatest impact of diarrhea on children's growth occurred in the first 3 years of life and, particularly, during the second half of infancy (6-12 months) and in the second year of life.

1.8. TREATMENT OF DIARRHEA

The goals of treatment are to maintain hydration, treat the underlying causes and relieve the symptoms of diarrhea. Rehydration and its correction of any electrolyte imbalance is critical in the treatment of diarrhea. Symptomatic relief is a second therapeutic goal [6].

Not all diarrheal episodes in the developing countries are associated with dehydration and, consequently, do not require rehydration therapy. However, promotion of the basic concept that diarrhea and vomiting are likely to results in life-threatening dehydration continues to be of great importance. This educational promotion should be aimed at all levels from families to doctors [3].

Oral rehydration therapy (ORT) was introduced in 1979 and rapidly became the cornerstone of the CDD programme (Control of Diarrheal Diseases). Consisting of the oral administration of sodium, a carbohydrate and water, ORT was potentially the most significant medical advance of the 20th century [56]. It has contributed substantially to reducing childhood deaths from diarrheal disease because it is extremely effective in treating acute watery diarrhea [57]. ORT, using the WHO formula, is suitable for the management of all types of dehydration [3].

ORS-WHO (oral rehydration salts) can be regarded as a universal, all-purpose, solution; but does not mean that is the optimal solution. However, it is important to have a single acceptable formula that can be recommended and promoted worldwide. ORS-WHO is an extremely safe therapeutic tool. More than two billion units of ORS have been administered without serious complications [3].

Symptomatic anti-diarrheal drugs are usually not recommended for the treatment of acute diarrhea in children [3, 6]. Antimicrobials are not effective in uncomplicated acute diarrhea and their use should be discouraged. In contrast, antimicrobials are indicated in dysentery, cholera, typhoid fever and diarrhea caused by parasites, such as *Giardia lamblia, Cyclospora* and *E. hystolytica* [3, 8].

One general principle of case management in acute diarrhea is dietary. It recommends that breast feeding must not be interrupted; feeding according to age should be restarted as soon as clinical signs of dehydration disappear, and be continued even if severe diarrhea persists. Adequate dietary management during and after diarrheal disease is very important in order to reduce or prevent the damage of intestinal functions induced by withholding foods; to prevent or decrease the nutritional damage caused by the disease; to shorten the duration of the disease; and to allow catch-up growth and a return to good nutritional condition during convalescence [3].

1.9. PREVENTION AND CONTROL OF DIARRHEA

The WHO' s CDD Programme and other organizations (UNICEF, USAID, etc) have given first priority the prevention of diarrheal deaths, rather than prevention of cases, and focused on promotion of ORT [3, 57]. It is estimated that ORT was used in about 69 % of all diarrheal episodes in developing countries [58].

ORT alone, however, has little impact on dysentery or on persistent and complicated diarrhea [57, 59], which currently account for over half of diarrhea deaths. A long-term, sustainable solution to childhood diarrheal disease must combine treatment with actions to eliminate diarrheal disease through prevention.

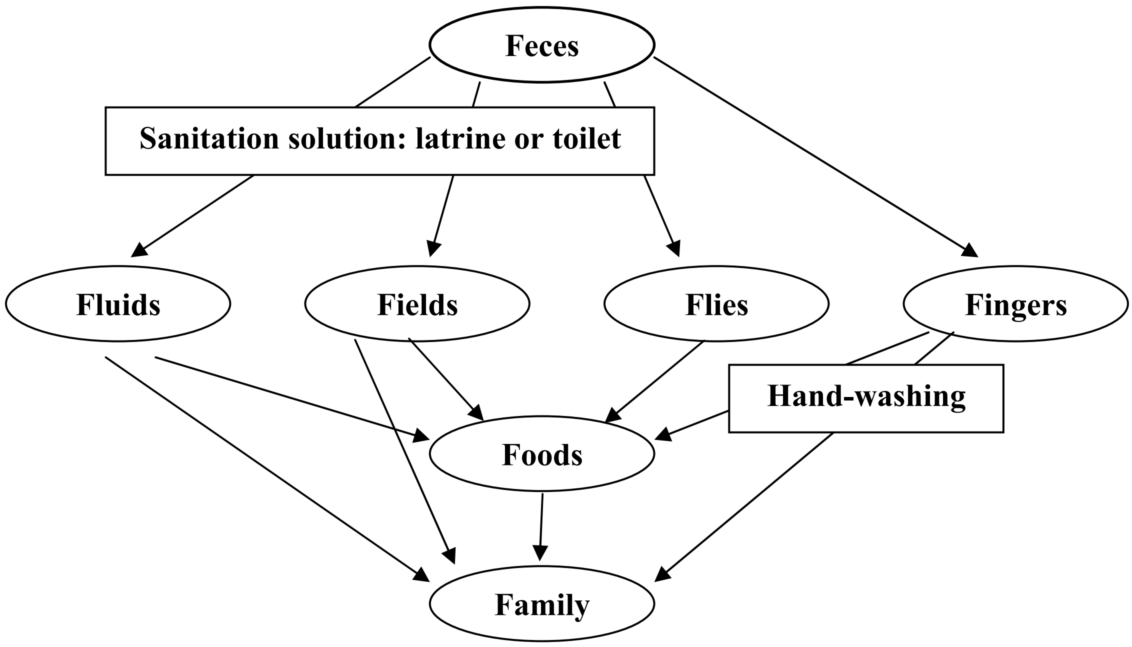


Figure1.1: ***Breaking the fecal-oral transmission cycle***.

It is estimated that 90% of the child diarrheal disease burden is the result of poor sanitation conditions and inadequate personal, household and community hygiene behaviors [60.] Therefore, understanding environmental and behavioral risk factors and their interactions is a prerequisite for devising effective preventive approaches [49].

Primary preventive interventions reduce environmental risk factors and high-risk behaviors for whole communities by interrupting the disease transmission cycle

(Fig.1.1). For diarrheal disease this means promoting changes in hygiene behavior to protect people from ingesting diarrheal disease pathogens and providing sanitation solutions to protect the environment from fecal contamination.

According to The Environmental Health Project [57] (supported by USAID) and T. Vesikari and B. Torun [3], strategies for comprehensive prevention and control of diarrhea include: good personal and domestic hygiene; use of safe water; improved nutrition; immunization; and effective case management. These strategies are summarized below:

Good personal and domestic hygiene:

- Effective hand-washing with a cleansing agent at critical times (after defecation, after handling children's feces, before feeding and eating, and before preparing food).
- Proper disposal of feces by using latrine and toilet.
- Adequate food hygiene, such as hygienic preparation and safe storage of foods.

Use of safe water:

- Use of drinking water from the safest source.
- Protection of drinking water from contamination at the source and in the home.

Improved nutrition:

- Breastfeeding (exclusively for 4-6 months and continuing to 1 year).
- Improved weaning practices.
- Growth monitoring.

Measles immunization: Of the existing vaccines, measles vaccine certainly has a potential in reducing mortality attributed to diarrheal disease since measles is associated with diarrhea in some 20 % of the cases [3].

Effective case management (home and health facility). Eight out of ten children who die do so at home, after having little or no contact with health facility staff. Therefore, implementing community IMCI is a priority for controlling diarrhea [61]. This strategy includes the following interventions:

- ORT
- Continuation of feeding during diarrhea.
- Intensive care for severe dehydration.
- Selective antibiotic therapy.

▪ Seeking medical care when needed.

Besides, female education, improvements of socioeconomic status and vitamin A supplementation may also play important roles in the prevention of diarrhea [3].

1.10. COUNTRY PROFILE

1.10.1. Background

Vietnam is located in South-East Asia, between latitudes 9 and 23 degree north, and longitude 106 degree east. It borders the Gulf of Thailand, Gulf of Tonkin, and South China Sea, alongside China, Laos, and Cambodia. The country has an area of 329,560 square kilometres, stretching over 1,600km along the eastern coast of the Indochinese Peninsula [62, 63].

Figure 1.2: ***the map of Vietnam***

Vietnam's population is of 82,689,518 inhabitants (July 2004 estimation) [62]. The population growth rate for Vietnam is 1.30%. The number of people aging 0-14 years accounts for about 29.4 % of the population, while the proportion of people 5-65 years and over 65 years of age are 65 % and 5.6 %, respectively. People who live in urban areas account for 20% of the population. Life expectancy of total population is 70.35 years (male 67.86 years and female 73.02 years). The infant mortality rate is 29.88 deaths/1,000 live births (2004 estimation) [62].

There are 56 ethnic groups in Vietnam, such as Kinh, Tay, Nung, Chinese, Hmong, Thai, Khmer, Cham, etc. Among them, the Kinh ethnic group is the majority, making up 85-90 % of the population.

Although the country is located in the tropical region, the climate is tropical only in central and southern Vietnam, with warm and humid weather all year round (22-35^{o}C). In the north, there is a distinct winter season due to cold inland winds. Usually, the winter is also the dry season for the entire country, but the rains are highly unpredictable owing to the influence of several monsoons [64]. Vietnam has a single rainy season during the south monsoon (May-September). Rainfall is abundant, with annual rainfall exceeding 1000mm almost everywhere. Rainfall is infrequent and light during the remainder of the year [65].

Vietnam is a poor country that has had to recover from the ravages of war and the rigidities of a centrally-planned economy. Substantial progress was achieved from 1986 to 1996 in moving forward from an extremely low starting point - growth averaged around 9% per year from 1993 to 1997. GDP (Gross Domestic Product) growth of 8.5% in 1997 fell to 6% in 1998 and 5% in 1999. Growth then rose to 6% to 7% in 2000-02 even against the background of global recession [62]. The GDP per capita was about US$ 470 in 2003 [66].

1.10.2. Health care system in Vietnam

Vietnam is divided into 4 administrative regions namely the North, the South, the Central and Highland, including 64 administrative provinces. Each province is divided into districts, and each district includes some communes. The health care network has been established from central to local areas. Ministry of Health is assigned to organize and manage health services all over the country. At local levels, provincial department of health, district medical centre and commune medical station are responsible for organizing, managing and providing health care services to the population in these areas. Structure of health care system can be summarized as follows:

- **National level:** Ministry of health (MOH); Medical Colleges; National Research Institutes; Central hospitals.

- **Provincial level:** Department of Health; Provincial hospital; provincial medical schools; specialized medical centres (such as preventive medicine centre, centre for tuberculosis control, etc).
- **District level:** district medical centre (including district hospital, team of hygiene and epidemiology), local general clinics.
- **Commune level:** commune medical station, village health workers; volunteers.

Over recent years the thrust of Vietnam's health sector strategy has emphasized active prevention, public service delivery at the "grass roots" level, the need to mobilize the entire society in support of improved health care, the expansion of health insurance cover, the value of traditional medicine, and the active participation of the private sector under the government's leadership [67].

For health spending, Vietnam has achieved remarkable results for a country that has limited public resources. Although Vietnam spends about 5-6 percent GDP on health care (both public and private expenditure), Vietnam has continued to make impressive progress in reducing infant mortality and under-five mortality rates. Progress in controlling vaccine-preventable diseases, such as measles, diphtheria and tetanus, has been rapid as well. Polio was completely eradicated in 1996 [67].

However, Vietnam's health sector has still some problems. Many new policy tools have been developed, including user fees, health insurance and health-care funds for the poor. These tools all focus on the financing of health, but still fail to merge into a coherent health financing system. And they coexist with tools organized by disease category, which operate under the form of National Health Programs (NHPs). There is little coordination between those programs, despite the fact that they often have the same target population (as in the case of tuberculosis and HIV/AIDS) and no mechanism in place to ensure that they are discontinued once their objectives are achieved [67].

Due to the lack of budget, the CDD program had been dismissed in 1999. Limited budget also leads to many difficulties, especially the inadequacy of the check-up system and shortage of hospital space. In most countries in the region, there is an average of 25 hospital beds for 10,000 people, whereas Vietnam only has 15 beds per 10,000 people. Medical insurance has covered only 21 % of the population[68].

In addition, income of health workers is particularly low, not corresponding to defined responsibilities and functions and not being able to promote the staff.

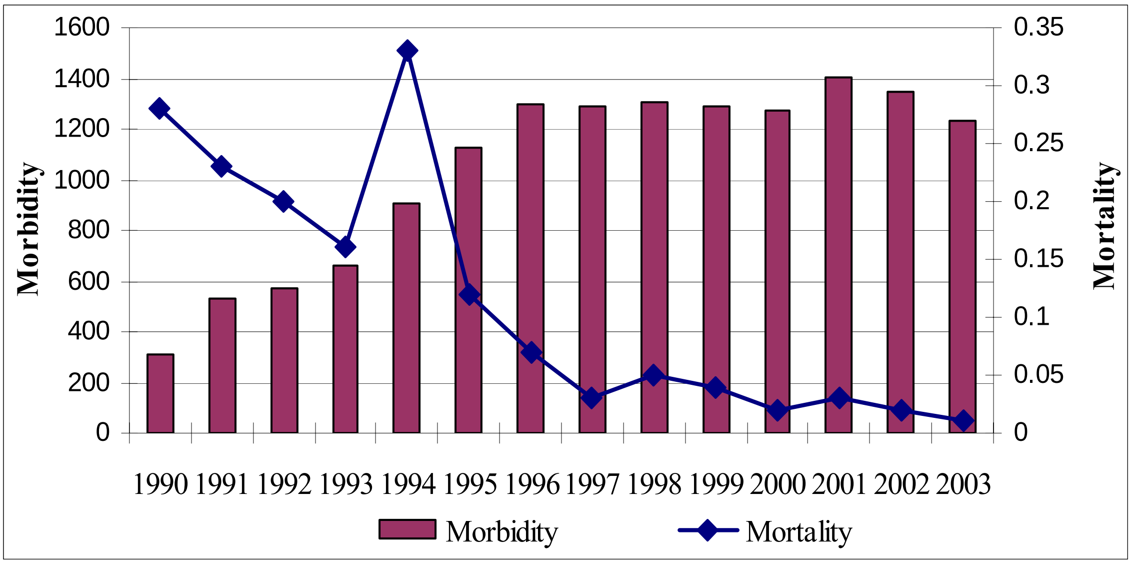
In terms of pharmaceutical industry, a few enterprises can produce drugs that reach good manufacturing practices (GMP) standard. Most of specific drugs have to be imported. Due to high prices, many low-income people cannot afford to access to these drugs.

1.10.3. Diarrhea in Vietnam

Crowded population, air and water pollution, poor sanitation, low hygienic practices and low socio-economic status pose a serious threat to public health in Vietnam. In terms of life expectancy adjusted for years lost to disabilities, Vietnam ranks 116 among 191 members of the WHO [67].

The morbidity of infectious disease remains high for both adults and children. Acute respiratory illness and diarrhea are leading causes of morbidity and mortality in children. The mortality rate among children less than 5 years of age was 42.2 deaths/1000 live births per year, of which diarrhea-related deaths accounted for 15.4% (2001 estimation) [69].

Figure 1.3: ***Morbidity and mortality of diarrhea per 100,000 populations in Vietnam between 1990 and 2003.***

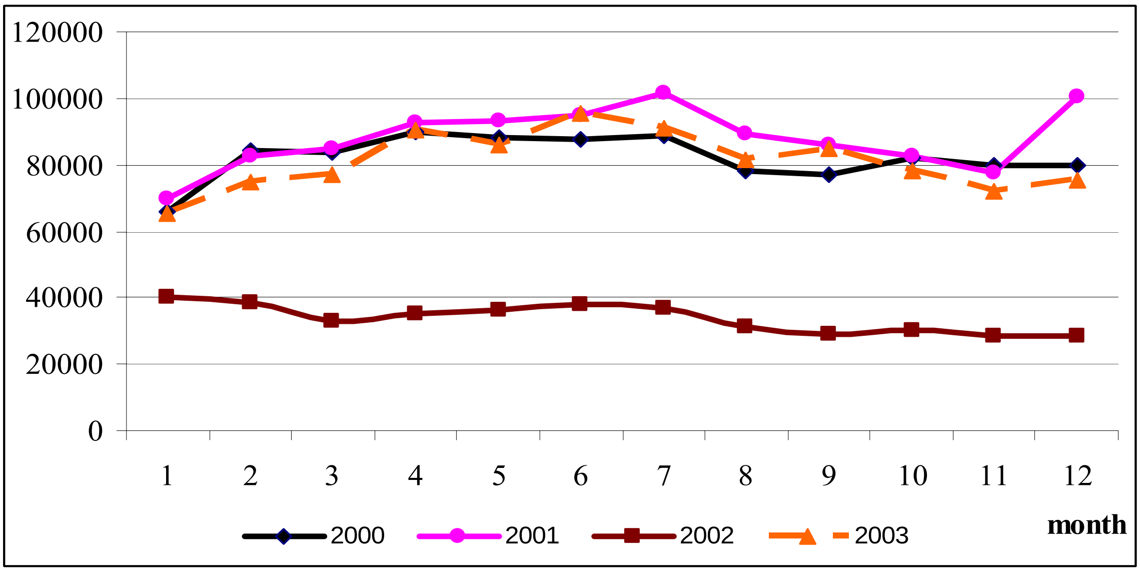


Source: *National Institute of Hygiene and Epidemiology, unpublished data*

As seen in fig 1.3, morbidity of diarrhea was relatively unchanged since 1996 (1,298.36/100,000 populations in 1996 and 1,236.17 in 2003), whereas mortality of diarrhea decreased considerably, from 0.33 in 1994 to 0.01/100,000 populations in 2003 [70]. However, the mortality could be underestimated due to the lack of surveillance information. Similarly, low morbidity and mortality of diarrhea in the period of 1990-1993 may be attributable to the weak surveillance system.

In Vietnam, according to some studies, the most common pathogens causing diarrhea among children under five are *rotavirus*, *E. Coli* (including entero aggregative *E. Coli*-EAggEC, entero toxigenic *E. Coli*-ETEC, entero pathogenic *E. Coli*-EPEC and entero invasive *E. Coli-EIEC*), *Shigella* (in which *Shigella flexneri* is the most common shigella serogroup), *Campylobacter jejuni*, *Vibrio cholera* and *Salmonella* [69, 70, 71, 72, 73].

Figure 1.4**: *Morbidity of diarrhea by month in Vietnam from 2000 to 2003***



***Source**: National Institute of Hygiene and Epidemiology (*NIHE*), unpublished data*

Figure 1.4 shows the morbidity of diarrhea by month in Vietnam between 2000 and 2003. The difference in the morbidity of diarrhea was insignificant between dry and rainy seasons nationwide, but in the north the higher prevalence of diarrhea has been observed in the rainy season (May-September) [70].

1.11. JUSTIFICATION OF THE STUDY

To effectively prevent diarrhea, it is imperative that the important risk factors associated with diarrhea should be identified first in communities through research. Over the world many studies have been conducted towards describing the epidemiology and risk factors for diarrheal disease among children less than five years of age. However, the local epidemiology of diarrhea in most rural areas of Vietnam has not been researched thoroughly. In addition, most studies in Vietnam have focused on a specific pathogen rather than identifying the most common pathogens of diarrhea among children in rural areas.

My study aimed to identify the most common pathogens of and local risk factors for diarrheal illness among children aged less than five years admitted to Dong Anh Hospital, Hanoi. Identification of pathogens and risk factors, and then recommendations of simple, immediate, and effective risk-reduction measures would help local health care services to reduce morbidity and mortality due to diarrhea among young children in the area.

CHAPTER 2

RESEARCH QUESTION, HYPOTHESIS AND OBJECTIVES

2.1. RESEARCH QUESTION

What are the most common pathogens of and potential risk factors for diarrheal disease among children under five years of age admitted to Dong Anh Hospital, Hanoi, Northern Vietnam?

2.2. HYPOTHESIS

We hypothesize that demographic, socio-economic, sanitation, drinking water related and food hygiene related factors are determinants of diarrhea occurrence among children less than five years of age in the district.

2.3. OBJECTIVES

General objective

To recommend suitable and effective measures to reduce morbidity due to diarrhea in the community.

Specific objectives

To identify risk factors for and the most common pathogens of diarrhea among children less than five years old admitted to Dong Anh Hospital.

CHAPTER 3

METHODS AND MATERIALS

3.1. STUDY SITE

The study took place in Dong Anh District, Hanoi, Northern Vietnam. Dong Anh is a suburban district locating in the north of Hanoi, at a distance of 40 kilometers from Hanoi city.

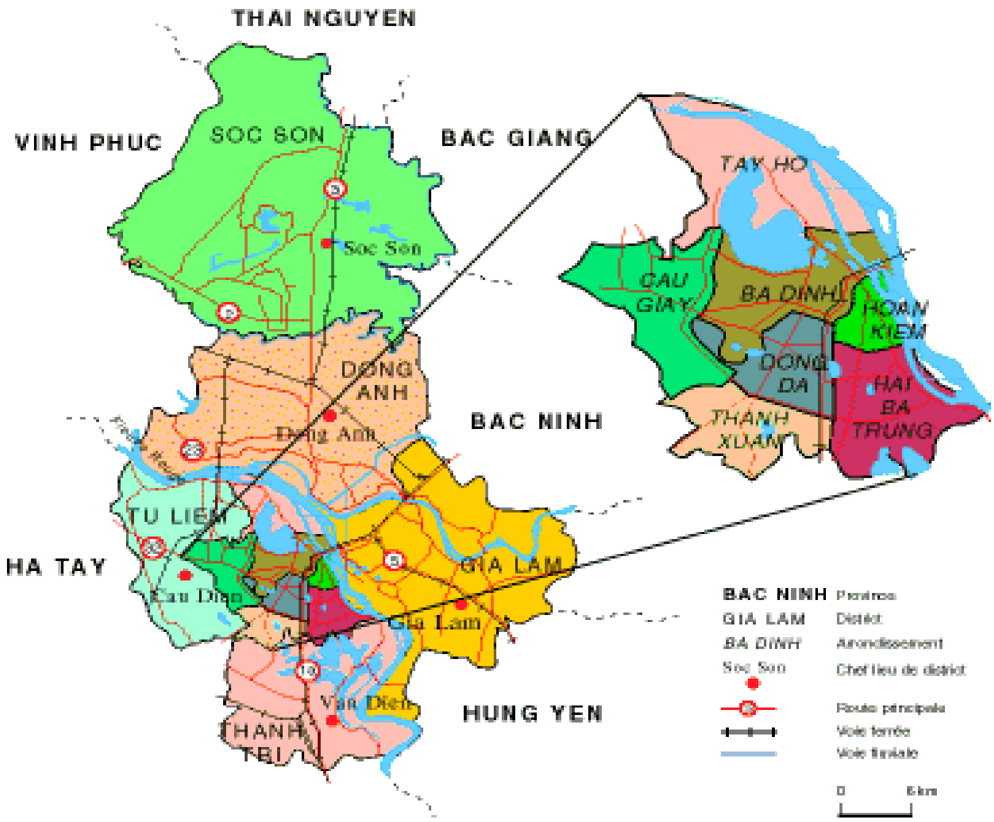


Figure 3.1: the map of Hanoi

Dong Anh District has an area of 182.3 square kilometers. Its population is of 283,309 people (2004 estimation). GDP per capita is approximately USD 200 in 2003. Like many areas in Northern Vietnam, it has a hot and rainy season (from May to September), and a cold season (from October to April). The average temperature is 23^0 C. The average rainfall is 1,500 to 2,000 mm. The humidity ranges around 80%.

Regarding the health sector in Dong Anh, a district general hospital with 180 beds is the referral hospital for the district. It also includes a team of hygiene and epidemiology. There are 25 commune medical stations established in the district, providing primary health care services to people at local level. Also, some private clinics, locating in Dong Anh Town, contribute to provide health care services to the population. According to data provided by Dong Anh Hospital's directorate, there were 2,912 children less than five years of age admitted to the hospital in 2004, of which 1,016 were diarrheal patients.

Dong Anh District was chosen for the study because it has specific characteristics of rural areas where existing water, sanitation and hygiene practices remain problems. In addition, no similar research, identifying the most common causes of diarrhea as well as risk factors associated with diarrhea among children less than five, has been conducted in the district before.

3.2. STUDY DESIGN: *a hospital-based case-control study*

Epidemiology is concerned with the distributions and determinants of disease frequency in human populations. The basic design strategies used in epidemiologic research can be broadly categorized according to whether such investigations focus on describing the distributions of disease or elucidating its determinant[74]. In the epidemiological approach to investigate associations between a disease and possible risk factors, cross-sectional, case-control and cohort designs can be employed [74, 75, 76, 77].

Cross-sectional study is a type of observational descriptive investigation, in which exposure and disease statuses are assessed simultaneously among individuals in a well-defined population. Thus, cross-sectional studies provide information on the prevalence and characteristics of a disease or other health outcomes of the population at a specified time. Such data can be of great value to public health administrators in assessing the health status and health care needs of a population [74]. Cross-sectional studies are less expensive and more expedient to conduct compared with analytic studies. Cross-sectional studies can be of some value in predicting future spread of certain disease through populations. Cross-sectional studies have one major advantage in that the studies are based on a sample of a major population and do not rely on individuals that present themselves for medical treatment.

However, cross-sectional studies have some disadvantages. These studies only represent those individuals who participated in the study. When used as a prevalence of disease assessment, cross-sectional studies are not too effective if the level of disease rates is very small. Since cross-sectional studies must consider prevalent rather than incident cases, the data obtained will always reflect determinants of survival as well as etiology. Recurrent conditions or diseases are not well represented as the condition or disease maybe dormant or inactive or at its peak when the study is conducted.

Cross-sectional studies establish association at most, not causality [77]. In most cross-sectional studies, the data can be used to describe characteristics of individuals with the disease and to formulate hypotheses, but not to test them. In one special circumstance, some cross-sectional studies can be considered as a type of analytic study and used to test epidemiologic hypothesis [75, 76]. This can occur only when the current values of the exposure variables are unalterable over time, thus representing the value present at the initiation of the disease. However, in this context, risk factors may be subject to alteration. To test the hypothesis stated above, this design was not chosen for the study.

There are two main types of observational analytic study that are used to investigate causal factors, namely cohort and case-control studies. In a ***cohort study,*** a group or groups of individuals are defined on the basis of presence or absence of exposure to a suspected risk factor for a disease. At the time exposure status is defined, all potential subjects must be free from the disease under investigation, and eligible participants are then followed over a period of time to assess the occurrence of that outcome. A principal advantage of cohort studies is that they are optimal for the investigation of the effects of rare exposures. With an uncommon exposure, it is unlikely that a sufficient number of exposed subjects could be identified in a case-control study even if the sample size was very large. Cohort studies can also examine multiple effects of a single exposure, thus providing a picture of the range of health outcomes that could be related a factor or factors of interest. Since the participants are disease-free at the time exposure status is identified, the temporal sequence between exposure and disease can be more clearly elucidated. Moreover, since in a prospective cohort study the outcomes of interest have not yet occurred at the time the study is begun, bias in the selection of subjects and ascertainment of exposure is minimized. Apart from above advantages, cohort studies allow the direct calculation of incident rates of the outcomes under investigation in the exposed and non-exposed groups [74].

For prospective cohort studies, since large numbers of subjects are required and followed up over time, usually for years, they can be extremely expensive and time consuming. Besides, validity of the results can be seriously affected by losses to follow-up [74, 76, 77]. For retrospective cohort study, the availability of adequate records is required. Due to these limitations, this design was not suitable for the study.

The second major type of observational analytic investigation is the ***case-control study,*** in which subjects are selected on the basis of whether they do (cases) or do not (controls) have a particular disease under study. The groups are then compared with respect to the proportion having a history of an exposure or characteristic of interest. The main outcome of a case-control study is an estimate of the relative risk of illness after various exposures. This estimate is given by the odds ratio (OR) [74, 75, 76].

Because of this design, case-control studies offer a number of advantages for evaluating the association between an exposure and a disease. In case-control studies, investigators could identify affected and unaffected individuals and look backward in time to assess their antecedent exposures rather than having to wait a number of years for the disease to develop. In addition case-control studies require fewer numbers of subjects than are required for prospective studies. Thus they can be conducted far more rapidly and less expensively than other analytic approaches. Moreover, because case-control studies select participants on the basis of their disease status, this design allows investigators to identify adequate numbers of diseased and none-diseased individuals. Consequently, this design is optimal for the investigations of rare diseases. Case-control studies also allow for the evaluation of a wide range of potential etiologic exposures that might relate to a specific disease as well as the interrelationships among these factors [74, 75, 76].

With respect to disadvantages, a case-control study is not an efficient design for the evaluation of a rare exposure; unless it is population-based, direct calculation of the incidence of disease in exposed and non-exposed groups is not possible; the temporal relationship between exposure and disease may be difficult to establish; and the greatest limitation of case-control studies is that they are more susceptible to bias than other analytic studies. In a case-control study, both the exposure and disease have already occurred at the time the participants enter into the study. As a result, this design is particularly susceptible to bias from the differential selection of either the cases or controls into the study on the basis of their exposure status as well as from differential reporting of exposure information between study groups based on their disease status[74].

There are some situations that can result in selection bias. The common element is that the relationship between the exposure and disease observed among those who participate in the study is different from that for individuals who would have been eligible to participate but were unwilling or not selected by the investigator. Similarly, if alternate controls are selected to replace those who initially chosen but could not be contacted or refused to participate, biased estimates could also result.

Recall bias occurs when individuals who have experienced a disease tend to think about the possible causes of their illness, and thus they are likely to remember their exposure histories differently from those who are unaffected by the disease.

Since the case-control design is particularly efficient, in terms of time and costs, and has particular utility in investigating the potential roles of multiple risk factors, this design was suitable for the study.

In the context of limited resources and study period, a community-base design was not feasible. Therefore, in this study, a hospital-based case-control design was used with both quantitative and qualitative methods. The definition and selection of cases, the selection of controls and the sources of information about risk factors and diarrhea were carefully considered to minimize or, preferably to avoid the bias that may arise when conducting the study. The field study was conducted in Dong Anh District, from July to December, 2005.

3.3. STUDY POPULATION

Study population included children less than five years of age admitted to Dong Anh Hospital from July to December, 2005. Since the children were too young at this age to be interviewed, the parents of recruited children instead were interviewed to identify risk factors for diarrhea.

3.4. SAMPLE SELECTION

3.4.1. Sample size

We could use the formula for the calculation of sample size in a case-control study [74], as follows:

$$\text{n (each group)} = \frac{(p_0 q_0 + p_1 q_1)(Z_{1-\alpha/2} + Z_{1-\beta})^2}{(p_1 - p_0)^2}$$

in which: p_1 is the proportion of exposure among cases.

p_0 is the proportion of exposure among controls.

$q_1 = 1 - p_1$; $q_0 = 1 - p_0$

$Z_{1-\alpha/2}$ is the value of the standard normal distribution corresponding to a significant level of alpha (e.g., 1.96 for a two-sided test at the 0.05 level).

$Z_{1-\beta}$ is the value of the standard normal distribution corresponding to the desired level of power (e.g., 0.84 for a power of 80 %).

If we know the value of p_1 and p_0 from previous studies or pilot studies, we could calculate the sample size of the study. However, we had inadequate data on such values. Therefore, we agreed to recruit 600 children into the study, including 200 cases and 400 controls. We feel this sample is big enough so that we could perform statistical analyses to identify major risk factors associated with diarrhea among children less than five years of age admitted to hospital in the district.

3.4.2. Sampling technique

Convenience sampling method was applied in the study because it is relatively easy and inexpensive to conduct. By this way, all children less than five admitted to Dong Anh Hospital were selected into the study. The period of time for selecting subjects was from 1st July to 31st December 2005. Over a time span of six months, we recruited 600 subjects into the study.

Selection of cases

All diarrheal patients less than five years of age admitted to Dong Anh Hospital from July to December 2005 were recruited into the study after their parents expressed the willingness to participate in the study. If the parent did not express the willingness to participate in the study, the child was not recruited. The willingness to participate by the parents was confirmed after spelling out to them the contents of the consent form (***see annex 2***).

To ensure that cases selected for the study represented a homogeneous entity, a strict definition of diarrhea was established.

A case was defined as a child less than 5 years of age having three or more loose, liquid, or watery stools or at least one bloody loose stool within 24 hours [73]. Persistent diarrhea was defined as diarrhea that began acutely and last for at least 14 days [3, 13].

In addition, the age of a child was verified by cross-examining the information provided in their health and vaccination cards, or simply by the confirmation of the mother.

Selection of controls

The selection of an appropriate comparison group is the most difficult and critical issue in the design of a case-control study. In this study, non-diarrheal patients less than five years of age admitted to Dong Anh Hospital between July and December 2005 were selected into the study. The recruitment of controls was carried out after their parents consented to participate in the study. Two controls were selected for each case recruited. Cases and controls were matched for sex and age. The age groups were defined as less than 1 year, 1 to 3 years, and 4 to 5 years.

The selection of controls who were hospitalized had some important practical and scientific advantages because they were easily to identified and readily available in sufficient number, thus minimizing the costs. Second, because they were hospitalized, their parents could be aware of antecedent exposures. This would help to reduce the potential for recall bias. Finally, like cases, the parents were more likely to be willing to cooperate than the parents of healthy children, thus minimizing bias due to nonresponse [74].

However, we faced a problem when selecting controls who were hospitalized. Since they were ill, and therefore differed from healthy children in some ways that may be associated with illness. Thus, the experience of the controls may not accurately represent the exposure distribution in the population from which the cases derived.

3.4.3 Inclusion and exclusion criteria

Inclusion criteria

All the children less than five years of age admitted to Dong Anh Hospital from July to December 2005 were eligible for the study. With respect to the parents of children recruited into the study, the mothers were suitable interviewees to provide adequate information about those children and other variables surrounding the children's environment because the mothers spent more time with their children than the fathers did.

Exclusion criteria

Children with following conditions were be rejected for the study: Those who were selected controls but had a history of diarrhea within the past two weeks; those who were cases but were diagnosed as intestinal diseases, irritable bowel syndrome, food intolerance and medication reaction; and those (both cases and controls) who were not resident in the district.

3.5. DATA COLLECTION

3.5.1. Pre-testing

The pre-testing was conducted on 5 mothers with diarrheal children and 10 mothers with non-diarrheal children less than five years of age admitted to the hospital. These people were not be recruited into the study after the selection of subjects process. The pre-testing was to check if they fetch the relevant answers to the questions to avoid information distortion.

After conducting pre-testing, some changes in the questionnaire were made. Since many of subjects' parents were farmers, we added one question to find out whether (or not) those farmers used manure as fertilizer. On breastfeeding status of children, in some cases mothers could not remember exactly when children had stopped being exclusively breastfed or when they had introduced other foods. Therefore we asked mothers whether (or not) their children had been exclusively breastfed in the first six month of life. To children under 6 months of age at the time the study conducted, we asked mothers whether (or not) their children had been exclusively breastfed to date.

3.5.2. Training of research assistants

Five research assistants were recruited for the study, two from Department of Microbiology-NIHE and three from Dong Anh Hospital. The questions and their meanings were thoroughly explained to the assistants. They were then instructed how to ask the questions and how to exactly report what the respondent answered. The assistants practised together to ensure a standardised way of collecting information.

In the process of collecting data, the principal researcher and the assistants checked data qualify after each field day of data collection. Corrections were made as necessary and possible.

3.5.3. Laboratory training

Training of laboratory technicians was held in Dong Anh Hospital. This ensured that stool sample collection and storage were complied with a standard protocol which has been applied in Enteric Pathogen Laboratory. At most 24 hours since being collected, fecal samples were transported to Department of Microbiology-NIHE for the identification of pathogens.

3.5.4. Data collection tool

The questionnaire

To avoid ambiguous answers, a questionnaire with clear and simple questions was designed. It was just the pre-tested questionnaire and had closed and open-ended questions. The questionnaire had seven sections: a section on demographic and socio-economic characteristics; a section on clinical data. This section was only used for the cases; a section on knowledge of diarrhea by the mothers; a section on sanitation and rubbish disposal; a section on hygiene related practices; a section on drinking water-related-practices; and a section on breastfeeding and vaccination status of the children. The questionnaire was developed in English language (*see annex 3*) and translated into Vietnamese, the only language for communication in the district.

3.5.5. Data collection techniques

Interviews

Face-to-face interviews based on the questionnaire were conducted on mothers of the children who were cases and controls recruited into the study. Interviews were conducted on the day of admission. Interviewers informed interviewees that participation in the study was voluntary. Interviewers explained the purpose of the study and asked interviewees for their permission to interview and collect stool samples from their children. Interviewees also were informed that the information they provided was handled as confidential and their individual answers would not be known, except by the interviewer and the coordinator of this study.

To those who were cases, a physician performed a physical examination and assessed the patient's dehydration status as mild, moderate or severe according to clinical signs. Information was also collected regarding antecedent exposure, diarrheal duration, stool frequency and treatment before admission.

Laboratory methods

Stool samples were collected from all cases recruited into the study immediately after their admission, and were then processed for bacterial, parasitological, and viral studies.

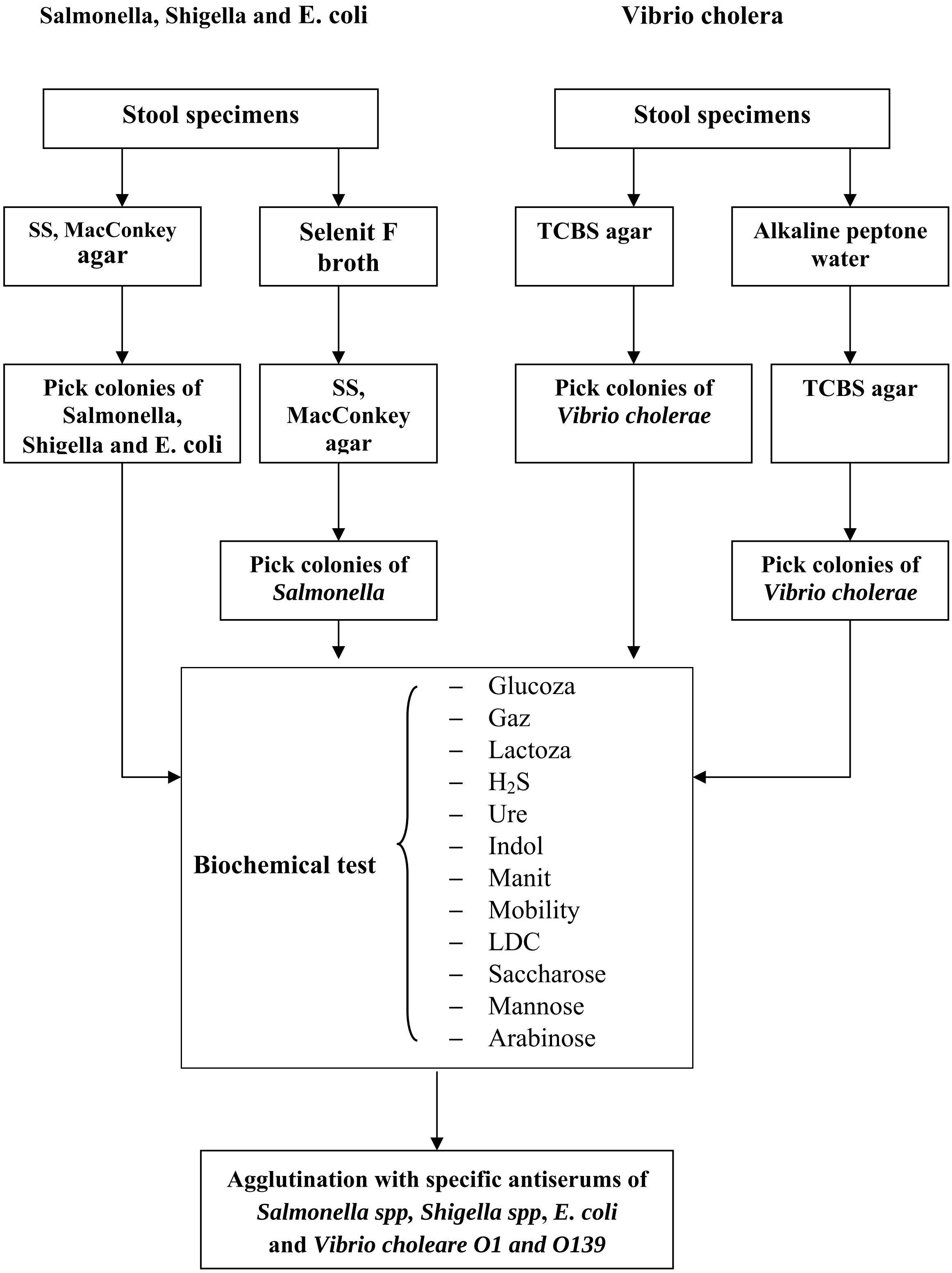
Parasitological studies: Each fecal sample was examined by direct microscopy in order to detect *Entamoeba histolytica, Giardia lamblia and Cyclospora cayetanensis*. Two methods were used: wet mount for amoeba, giardia and cyclospora; and Ziehl-Neelsen carbolfuchsin staining of formalin concentrates for identification of c*yclospora* only. Wet mount and stained smear was examined under 400x magnifications.

Bacteriological studies: Enteric pathogens were investigated by culture. Fresh stool samples were inoculated on *Salmonella-Shigella* (S-S) agar and MacConkey agar. For *Salmonella* enrichment, stool samples were inoculated in Selenite-F broth, incubated at 37 ^{0}C for 18 hours and then subcultured on S-S agar. All plates were examined and suspected colonies of enteropathogens were identified by standard biochemical methods. Further identifications were done by specific antiserum of *Salmonella spp, Shigella spp*, and *E. coli*.

For the identification of *Vibrio cholera*, stool samples were inoculated on thiosulfate citrate bile salt sucrose agar (TCBS). To enrich *Vibrio cholerae*, stool samples were inoculated in alkaline pepton water (APW), incubated at 37^{0}C for 3-6 hours and then subcultured on TCBS agar. Suspected colonies of *Vibrio cholerae* were identified by standards biochemical method and specific antiserum of *Vibrio cholerae O1* and O139.

Figure 3.2: **Procedures for isolation of *Salmonella, Shigella, E. coli* and *Vibrio cholera* from stool specimens**

(Issued by Enteric Pathogen Laboratory - NIHE)



Virological studies: regarding viral pathogens, due to limited resources, we detected only rotavirus, the most common virus causing diarrhea in children. *Rotavirus* was detected by using an enzyme immuno assay (EIA) test kit. This kit, made by Poliovac (Poliomyelitic vaccine research and production, Vietnam) and Denka Sciken Company (Japan), includes: Rotavirus monoclonal antibody – coated - plate that encompasses wells; positive control; sample diluent (this diluent is also negative control); Enzyme conjugate; substrate B; Phosphate Buffered Saline-tween (PBS); and stop solution. The EIA test procedure includes following steps:

Step 1:

- Dilute fecal samples in the diluent. Dilution ratio was 10 %.
- Add 100 µl diluted samples to each well and shake the pale for several seconds by plate shaker.
- Add 100 µl positive control and 100 µl negative control into 2 separated wells.
- Cover the plate with an aluminium foil and incubate the plate at room temperature for 1 hour.

Step 2:

- Remove diluted fecal samples from wells and wash wells by 200 µl of PBS tween. Repeat the washing twice (total 3 times).
- Invert the plate, slap firmly on a stack of clean paper towels to remove any residual solution from wells.
- Add 100 µl of enzyme conjugate into each wells. Shake the plate for several seconds.
- Cover the plate with the aluminium foil and incubate the plate at room temperature for 1 hour.

Step 3:

- Remove solution from wells and wash wells by 200 µl of PBS tween. Repeat the washing 4 times (total 5 times).
- Invert the plate, slap firmly on a stack of clean paper towels to remove any residual solution from wells.
- Add 100 µl subtrate B into each well. Shake the plate for several seconds.
- Cover the plate with the aluminium foil and incubate the plate at room temperature for 30 minutes.

Step 4:

- Add 100 μl stop solution into each well and shake the plate for some seconds.

Within 30 minutes after completing the above steps, we observed the colour of solution in the wells. Solution in the well which was added with positive control appeared blue colour. Solution in the well with negative control appeared white colour. Based on the colour in the wells we identified which samples were positive to rotavirus.

3.6. VARIABLES AND DEFINITIONS USED IN THE STUDY

Two types of variables were used in the study, namely dependent and independent variables.

3.6.1. Dependent variables

The study has one dependent variable which is diarrhea. Acute diarrhea is defined as three or more, loose, liquid, or watery stools or at least one bloody loose stool within 24 hours. Persistent diarrhea is defined as diarrhea that begins acutely and lasts at least 14 day.

With respect to the section on clinical data employed to the cases only, variables, like duration of diarrhea; stool frequency per day; whether (or not) the blood presents in stool; whether (or not) vomiting occurs; patient's dehydration status; are merely used to describe clinical signs/symptoms of diarrhea, or complication caused by diarrhea.

3.6.2. Independent variables

The independent variables in the study are regarded as the potential risk factors for diarrhea among children less than five years based on the literature review, including demographic, socio-economic factors, knowledge of diarrhea stated by the mothers, sanitation-, hygiene-, and water-related factors, and the child's breastfeeding and vaccination statuses.

Regarding the section on clinical data, the child's exposure antecedently to any diarrheal patient or contaminated-suspected food sold by street vendors in the last 7 days, were also regarded as independent variables.

Demographic and socio-economic factors included age of both the child and mother, level of mother's education, marital status and ethnicity of mother, total number of children per mother, birth order, and occupation of the parents and economic status of the family. Economic status of the family was relatively assessed through using the following variables: Total income of the family per month, number of rooms and number of people living in the house, and the possession of some household facilities and some livestock as well. Based on the fact in Vietnam's rural areas, the economic status of a family was assessed as follows: well-off or poor. A family was said to be well-off if its income was over 500 NOK per month and it had a house with at least one of the following items: motorbike, refrigerator; or both television and compact disk player. A family did not satisfy the above conditions was said to be poor.

Sanitation and rubbish disposal: a number of questions was used to find out whether (or not) they have a latrine, how they defecated if they did not have a latrine; type of latrine; hygienic status of the latrine; and number of people using the latrine. They was also be asked whether (or not) their children could use the latrine on their own, if their children could not, to state where their children defecate, how they disposed the feces; what care they gave to their children after going to toilet; and where waste garbage and water was disposed of.

Hygiene related factors: mothers were asked if their children could feed on their own, and if their children could, the child ate with a spoon or with his/her hands, whether (or not) mothers washed their children's hands before eating.

Questions were asked to find out if mothers wash their hands at 4 critical times (after going to toilet; after helping their children defecate; before eating and feeding their children; and before preparing food for their children). If the answer was yes, they were asked specifically with what they washed their hands.

Mothers was also be asked if cooked foods were stored; how and how long the foods were stored for later use; whether (or not) the left-over foods were heated before use; how they cleaned utensils for feeding their children; and whether (or not) they often bought foods sold by street vendors for their children. In addition, questions on hygienic status of kitchen, the presence of flies or domestic animals in kitchen were asked in the interview.

Water related practices: variables, like water sources used for domestic needs, treatment given to water before carrying home, types of utensils used for storing water, types of water used for drinking, and the place mothers bath their children, were used in the study.

Knowledge of diarrhea: mothers were asked about whether (or not) they know about diarrhea. If they did, they were asked to state some of the signs/symptoms of diarrhea. They were asked if they knew some causes of diarrhea, and what spreads diarrhea. They were also be asked if they know how diarrhea could be prevented, if they did, to mention some of the ways they could remember and how they had come to know about these. Lists on means of spread also included unsafe drinking water, unsafe fecal disposal, careless disposal of garbage, not coving foods to avoid flies. Also, lists on means of prevention included disposing of stools in the latrine, washing hands at the four critical times and the use of water for drinking.

Breast feeding and vaccination statuses: mothers were asked if their children were still being breastfed or weaned. In case of breastfed children, several questions were used to find out if their children have been exclusively breastfed to the day of interview or not; how long their children have been introduced to other foods; whether (or not) mothers know about benefits of adequate breastfeeding in reducing infections in children.

Variables to assess the child's vaccination status include types of vaccine the child has been vaccinated against measles and some other diseases. The interviewers could assess the child's vaccination status by asking the mother or checking information in the health and vaccination cards.

3.7. DATA HANDLING AND DATA ANALYSIS

Data collected were entered into a computer in the Epi-data software, version 3.1 and the SPSS 12.0 software for the analysis.

Numerical variables like age of child and mother, number of siblings of the child, etc, were entered as they were without being recoded. As regards categorical variables like sex, mother's marital status, parents' occupation, etc, were entered after being recoded.

Economic status of the family was categorised in 2 groups namely well-off and poor. The criteria to categorize were mentioned in section 3.6.2.

Categorization of knowledge of diarrhea was in four groups, namely very good knowledge, good knowledge, poor knowledge and no knowledge. The categorization was based on mothers' knowledge regarding signs, causes, means of spread and prevention of diarrhea.

For signs of diarrhea, mothers who mentioned a minimum of 3 correct signs, less than 3 signs, no correct sign or failed to point out any sign at all were put respectively into groups of having very good knowledge, good knowledge, poor knowledge and no knowledge.

For causes of diarrhea, mothers who mentioned at least 2 correct causes, less than 2 causes, no correct cause or failed to mention any causes were said respectively to have very good knowledge, good knowledge, poor knowledge and no knowledge.

Likewise, for means of spread and prevention of diarrhea, if mothers' mentions covered at least 3 areas of sanitation, hygiene and water, they were said to have very good knowledge, less than 3 above areas having good knowledge, none of the 3 areas having poor knowledge. Those that did not say anything were said to have no knowledge.

Those not having any correct answer in all of the 4 above-said knowledge of diarrhea were considered to have no knowledge.

Bivariate analysis was performed to identify factors associated with diarrhea by calculating the OR and 95 % CI, with the statistical significance that was set at the level $p < 0.05$. Multivariate analysis was then used to find out whether (or not) the factors, which were significantly identified in bivariate analysis, remain independently associated with the risk of diarrhea [75].

3.8. RESEARCH TEAM

In the collaboration between Department of Microbiology-NIHE and Dong Anh Hospital, a research team was established to conduct the study, including the principal researcher, 2 researchers from Department of Microbiology-NIHE and 3 staff from Dong Anh Hospital.

3.9. ETHICAL CONSIDERATION

The researchers explained the purpose and benefits of the study to the subjects and asked them for their permission to interview and collect specimens. Participation in the study was totally voluntary. Participants were not forced or persuaded to participate in the study. Even those who initially accepted to participate were free to withdraw in the course of the study if they did not wish to continue. The researchers had to guarantee the anonymity of the participants and the confidentiality of the information they provided.

Since the study was conducted by asking mothers of children recruited to gather information and collecting stool samples from the cases, the conduct of the study did not pose any health risk to the participants.

The study had to be approved by the Department of International Health, Faculty of Medicine, University of Oslo-Norway and the Ministry of Health (MoH)-Vietnam. The project was submitted to the two bodies for ethical clearance. Also, permission from Dong Anh Hospital's directorate and local authorities was obtained before conducting the study.

The study's results will help local health care services employ simple, immediate, and effective measures in order to decrease morbidity and mortality due to diarrhea among young children in the area.

2.10. TIME TABLE

Month	Works
June 2005	
13 - 17	- Visit and work with Dong Anh Hospital's directorate. - Recruit researcher assistants. - Meet and discuss with all members of the research team to reach a consensus on the study's schedule. - Train interviewers. - Train laboratory technicians working in Dong Anh Hospital.
20 – 30	- Conduct the pre-testing. - Modify the questionnaire as necessary.
July	- Collect data - Work in laboratory. - Arrange meetings for the research team to discuss and decide solutions to problems occurring in the process.
August	- Collect data - Work in laboratory
September	- Collect data - Work in laboratory
October	- Collect data - Work in laboratory
November	- Collect data - Work in laboratory
December 2005	- Collect data - Work in laboratory - Enter data into the computer
January 2006	- Data compilation and analysis
February - June 2006	- Write thesis - Defend thesis

CHAPTER 4:

RESULTS

4.1. Characteristics of the study sample

A total of 600 children under five years of age, including 200 cases and 400 controls, were recruited into the study after meeting the inclusion criteria. Interviews based on questionnaire were conducted with 600 mothers. For the group of cases, 200 stool samples were collected and transported to Enteric Pathogen Laboratory - NIHE to identify pathogens causing diarrhea.

4.1.1. Distribution of cases by month

Among 200 cases, 45 (22.5 %) cases were recruited in July, 2005; 38 cases in August (19 %); 20 cases (10 %), 42 cases (21 %) and 41 cases (20.5 %) in September, October and November, respectively. There were only 14 cases (7 %) recruited in December because the number of 200 cases was reached on 15th Dec, 2005. Data on distribution of cases by month is shown in figure 4.1.

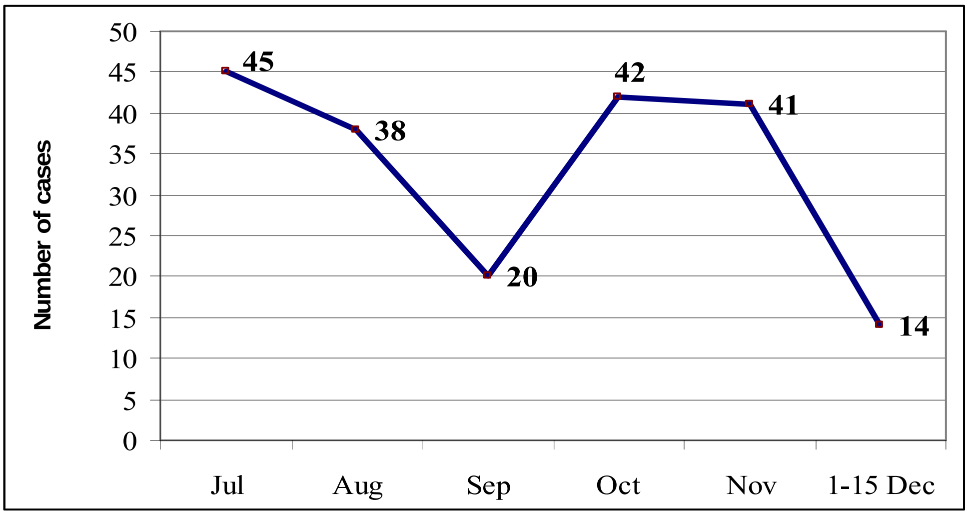


Figure 4.1: Distribution of cases by month

4.1.2. Distribution of cases by village

With regard to geographic distribution of cases, of the total 25 villages and 1 town in Dong Anh District, 20 villages and Dong Anh Town were reported having cases. Detailed number and percentage of cases occurring in the above-mentioned villages are shown in table 4.1.

Table 4.1: Geographic distribution of cases by village

Villages	Frequency	Percentage
Bachong	9	4.5
Coloa	8	4.0
Daimach	4	2.0
Donghoi	6	3.0
Ductu	15	7.5
Haiboi	4	2.0
Kimchung	4	2.0
Kimno	4	2.0
Lienha	23	11.5
Mailam	8	4.0
Namhong	8	4.0
Nguyenkhe	9	4.5
Dong Anh town	5	2.5
Thuylam	14	7.0
Tienduong	20	10.0
Uyno	9	4.5
Vanha	21	10.5
Vannoi	5	2.5
Viethung	13	6.5
Vinhngoc	8	4.0
Xuancanh	3	1.5
Total	**200**	**100.0**

4.1.3. Demographic and socio-economic characteristics

As seen in table 4.2 and figure 4.2 below, of the total 200 cases recruited into the study, there were 124 males and 76 females. The number of males was higher than females in almost all age groups.

Table 4.2: Distribution of cases by sex and age group

Age group	Frequency			Percentage
	Male	*Female*	*Total*	
Under 6 months	15	6	**21**	10.5
6 – 11 months	45	24	**69**	34.5
12 – 23 months	48	31	**79**	39.5
24 – 36 months	15	15	**30**	15.0
37 – 59 months	1	0	**1**	0.5
Total	124	76	**200**	100 %

The minimum age of cases was 2 months; the maximum age was 48 months representing an age range of 46 months. The mean age was 14 months. Cases were mostly children less than 2 years of age. Children aged less than 1 year had 90 cases, making up 45 % of the total. The number of cases in the 1-to-under 2-age group was 79 (39.5 %).

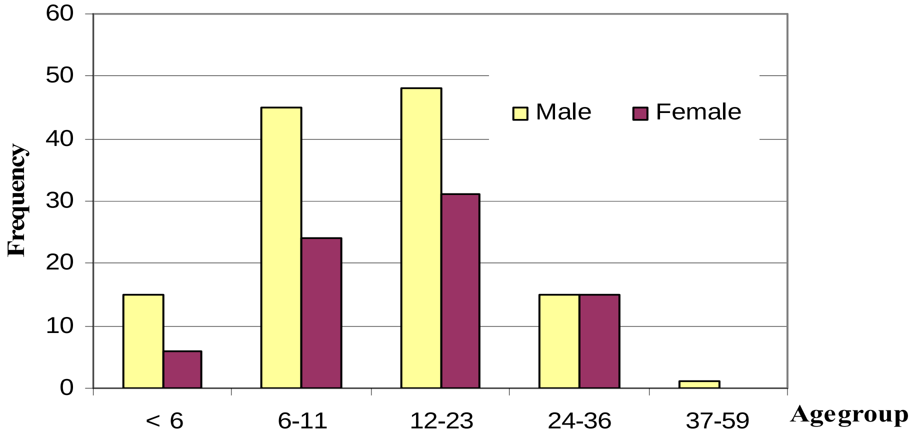


Figure 4.2: Distribution of cases by sex and age group

Along with matching variables (sex and age group), some of other demographic and social characteristics of cases and controls, such as mean age of children and mothers; ethnic group as well as family size, are shown in table 4.3.

Table 4.3: Other demographic and social characteristics of cases and controls

Characteristics		**Study group**	
		Cases (*n=200*)	**Controls (*n=400*)**
Sex:	Male	124 (62 %)	248 (62 %)
	Female	76 (38 %)	152 (38 %)
Age group:	Under 1 year	90 (45 %)	180 (45 %)
	1-3 years	109 (54.5 %)	218 (54.5)
	4-5 years	1 (0.5 %)	2 (0.5 %)
Mean age (month)		13.7	14.3
Mean age of mother (year)		27.6	26.2
Ethnic group:	Kinh	200	400
Family size	Over 5 people	35 (17.5 %)	117 (29.25 %)
	≤ 5 people	165 (82.5 %)	283 (70.75 %)

With regard to other variables in the demographic and socio-economic section that were seen as potential risk factors associated with diarrhea, such as level of mother's education, marital status, parents' occupation, number of siblings of the child, number of people sharing a room; and assessment of the family's economic status, would be analyzed and discussed in sections of bivariate and multivariate analyses.

4.1.4. Knowledge of diarrhea among mothers

Results of knowledge of diarrhea among mothers in 2 groups, based on categorization criteria in section 3.7, are summarized in table 4.4 below.

Table 4.4: Knowledge of diarrhea among mothers

	Very good N (%)	**Good** N (%)	**Poor** N (%)	**None** N (%)	**Total**
Knowledge of signs					
Case group	35 (17.5)	154 (77)	0	11 (5.5)	200
Control group	132 (33)	259 (64.75)	0	9 (2.25)	400
	p< 0.001	*p< 0.001*		*p=0.04*	
Knowledge of causes					
Case group	27 (13.5)	160 (80)	2 (1)	11 (5.5)	200
Control group	59 (14.75)	332 (83)	0	9 (2.25)	400
	p=0.7	*p=0.4*		*p=0.04*	
Knowledge of spread					
Case group	2 (1)	140 (70)	47 (23.5)	11 (5.5)	200
Control group	17 (4.25)	358 (89.5)	16 (4)	9 (2.25)	400
	p=0.03	*p<0.001*	*p< 0.001*	*p=0.04*	
Knowledge of prevention					
Case group	31 (15.5)	127 (63.5)	31 (15.5)	11 (5.5)	200
Control group	29 (7.25)	359 (89.75)	3 (0.75)	9 (2.25)	400
	p=0.001	*p< 0.001*	*p< 0.001*	*p=0.04*	

Of 600 mothers participated in interviews, only 11 mothers (5.5 %) in the case group and 9 mothers (2.25 %) in the control group had no knowledge of diarrhea. Using *Chi-square* test, the difference between 2 groups in terms of having no knowledge of diarrhea was found statistically significant (p=0.04).

For knowledge of diarrhea signs and causes, mothers having very good and good knowledge made up very high percentage.

However, 47 mothers (23.5 %) and 31 mothers (15.5 %) in the case group had poor knowledge of diarrhea spread and prevention, respectively. In the control group, 16 mothers (4%) had poor knowledge of diarrhea spread and only 3 mothers (0.75%) had no knowledge of prevention.

In almost all categorized groups, the number of mothers who had very good and good knowledge of diarrhea in the control group was higher than that in the case group. On contrary, number of mothers having poor and no knowledge of diarrhea in case group was higher compared with control group. The above-mentioned differences between 2 groups is statistically significant ($p< 0.05$)

About how mothers acquired knowledge of diarrhea, among 200 mothers in the case group, 117 (58.5%) had come to know through television, 24 (12%) through reading, 13 (6.5%) through radio, 66 (33%) through their attendance in medical facilities, 74 (37%) through village health workers, and 25 (12.5%) through interactions with their friends or other people.

For mothers in the control group, 162 (40.5%) had come to know through television, 77 (19.25%) through reading, 65 (16.25%) through radio, 143 (35.75%) through their attendance in medical facilities, 206 (51.5%) through village health workers, and 38 (9.5%) through interactions with their friends or other people.

Independent variables in other sections, such as sanitation, hygiene and water-related-practices; and breastfeeding status of the child, also were analyzed in bivariate and multivariate analyses.

4.2. Clinical history and manifestation

For the case group, watery stool frequency ranged from 3 times to 20 times per day, with a mean of 6 times per day. There were 104 reported cases of 3-5 watery stools per day, accounting for 52 % of the total. 86 cases (43 %) had daily stool frequency of 6 – 10 times. Stool frequency of over 10 times per day was reported in 10 cases (5 %).

Mean number of days with diarrhea was 2.4. There were 176 cases (88%) suffering diarrhea from 1-3 days; 20 cases (10 %) had diarrhea that last 4-6 days. Diarrhea lasting 7 -10 days was reported in only 4 cases (2 %). There was no case of diarrhea lasting more than 2 weeks.

Blood in stool were reported in 18 cases (9 %). Vomit occurred in 88 diarrheal children (44 %), with a mean of 3 times per day.

15 cases (7.5 %) were reported to have contact with diarrheal patients and 11 cases (5.5%) had eaten foods sold by street vendors for the last 7 days.

There were 26 cases (13 %) receiving oral antibiotics before their admission. After admission to Dong Anh Hospital, 88 (44 %) cases were diagnosed to be in a mild dehydration situation. 106 (53 %) and 6 children fell in the moderate and severe dehydration situation, respectively.

Fever also was reported in 86 cases, including 47 slight (37.5 - below 38.0 ^{0} C), 28 moderate (38.0 - below 39.0 ^{0} C) and only 11 high fever patients (from 39.0 ^{0} C upwards).

After admission, all 200 diarrheal received rehydration therapy (oral or/and transfuseble). 193 cases, making up 96.5 %, were prescribed antibiotics. Other medications, such as antipyretics and vitamins, also were used for treatment.

4.3. Bivariate analysis of potential risk factors associated with diarrhea

In bivariate analysis, based on findings that were presented with matched odds ratio (MOR), 95 % confidence interval (CI) and p value, we found 19 factors significantly associated with diarrhea. Detailed results of bivariate analysis are shown in table 4.5 (page 57 - 60).

4.3.1. Socio-economic factors

Among 600 mothers recruited into the study, there were 152 mothers (76%) in case group and 238 mothers (59.5%) in control group having only primary education. We have MOR = 2.2, 95 % CI 1.5 – 3.2, and p value < 0.001. We say that the risk of diarrhea in children whose mothers had only primary education is 2.2 times higher than those whose mothers had higher level of education.

Comparing with primary education, higher levels of education of mothers, such as secondary and bachelor, are associated with decreased risk of diarrhea. In other words, OR decreased inversely in proportion to higher level of education of mothers (OR=0.6; 95%CI: 0.6 – 0.95; p=0.002 and OR=0.3; 95% CI 0.1 – 0.6; p<0.001, respectively)

When using logistic regression for this variable, considering primary education as reference category, we have the same results (*see table 4.6*). The odds against diarrhea in children whose mothers had secondary education is 1.7 times higher compared to those whose mothers had primary education (OR=1.7, 95% CI 1.1 – 2.6; p=0.02). The odds against diarrhea in children whose mothers graduated from college or university is 3.6 times higher compared to those whose mothers belong to the reference group (OR=3.6; 95% CI 1.9 – 6.9; p<0.001)

Table 4.6: Results of logistic regression on mothers' level of education

Variables in the equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% CI for EXP(B)	
								Lower	Upper
Step 1(a)	Education			18.266	2	.000			
	Education(1)	.511	.222	5.316	1	.021	1.668	1.080	2.576
	Education(2)	1.286	.330	15.203	1	.000	3.619	1.896	6.909
	Constant	1.048	.128	67.083	1	.000	2.851		

a. Variable(s) entered on step 1: education.

There were 4 cases (2%) and 9 controls being brought up by only their mothers, showing no association between diarrhea and marital status (OR=0.9; 95% CI 0.2 – 3.2; p=0.9).

When we used mean age of mothers as a cut-off point to categorize mothers into 2 groups: a group of mothers under 26, and another group of mothers from 26 upwards, we also observed no significant association between diarrhea and mothers' age (OR=0.8; 95% CI 0.6 – 1.2; p=0.2)

Parents' occupation as farmer was found to increase the risk of diarrhea in children compared to employed and self-employed job. [OR=2.5; 95% CI 1.7 – 3.7; p<0.001 (to farmer mothers); OR=3.0; 95% CI 2.1-4.3; p<0.001 (to farmer fathers)].

With regard to economic status, based on the definition of low economic status, 83 families (41.5%) in case group and 91(22.8%) in control group were in poor economic status. Statistic results showed that low income in particular and poor economic status in general is associated with increased diarrhea (OR=2.4; 95%CI 1.7 – 3.4; p<0.001).

62 cases and 184 controls were the only child in the family. When taking this figure as reference category, having one sibling or more was found to be a risk factor for diarrhea (OR=1.9; 95% CI 1.3 – 2.7; p<0.001).

When using logistic regression, birth order of the child was not significant to the risk of diarrhea.

4.3.2. Sanitation factors

When being asked about latrine type, 130 families (65%) in case group used old-type latrines (including two-compartment and one-compartment latrines) comparing to 197 (49.3%) in control group (OR = 1.9, 95% CI 1.3 – 2.7, p< 0.001).

Among potential risk factors in the section of sanitation and rubbish disposal, we found 3 factors more that were associated with diarrhea, namely irregular latrine cleaning (1-3 times per week. OR=3.1; 95% CI 2.1 – 4.7; p< 0.001); latrine-sharing among more than 5 people (OR=2.3; 95% CI 1.3 – 4.1; p=0.002); and using stool as fertilizer (OR=3.9; 95% CI 1.5 – 9.9; p=0.002).

4.3.3. Hygiene – related – factors

For the practice of washing hands, there were 6 factors found to increase the risk of diarrhea, including: No hand-washing for the child before eating (OR=1.5; 95% CI 1.2 – 2.2; p=0.014); irregular hand-washing by mothers after going to toilet(OR=6.0; 95% CI 3.5 – 10.3; p<0.001); irregular hand-washing by mother after helping the child to defecate (OR=2.3; 95% CI 1.6 – 3.3; p<0.001); No hand-washing by mother before feeding the child (OR=7.7; 95% CI 3.2- 18.1; p< 0.001); No hand-washing by mother before preparing foods for the child (OR=5.1; 95% CI 2.6 – 9.8; p< 0.001); hand-washing by mother with water only instead of with soap and water (OR=2.9; 95% CI 1.9 - 4.3; p<0.001).

When taking not storing foods for later use as reference category, storing foods in larder and disk-cover were found to increase the risk of diarrhea (OR=5.3; 95% CI 3.2-8.7; p<0.001; and OR=2.6; 95% CI 1.5 – 4.6; p<0.001, respectively). If we considered both the 2 above-mentioned ways as unsafe storage of foods, we had 103 families (51.5%) in the case group and 90 families (22.5%) in the control group. Statistical findings showed the unsafe food storage was also a risk factor (OR=3.7; 95% CI 2.5 – 5.3; p < 0.001).

Another food-related-behavior was found associating with diarrhea. That was buying foods for children from street vendors (OR=1.6; 95% CI 1.04 – 2.5; p=0.03).

Daily kitchen-cleaning was seen as protective against diarrhea. When being asked, 113 mothers (56.5%) in the case group and 75 mothers (18.8%) in the control group admitted irregular kitchen cleaning (2-3 times per week or every week). We saw that OR increased inversely in proportion to the less regular cleaning of the kitchen: OR=3.7, 95% CI 2.5 – 5.6; p<0.001 (2-3 times per week); and OR=32.1; 95% CI 12.4 – 83.5; p< 0.001 (every week). In other words, irregular kitchen cleaning increased the risk of diarrhea (*see table 4.5*).

Some other variables, such as presence of flies and animals in kitchen; keeping animal in kitchen overnight, were not significantly associated with diarrhea (p>0.05).

4.3.4. Water – related – factors

Among water – related – factors, infrequent cleaning/emptying of storage container before refilling it with fresh water was found associated with increased risk of diarrhea (OR=4.1; 95% CI 2.8 – 6.1; p<0.001).

All 600 mothers stated that their families used boiled-water for drinking and all their children were bathed in bath room or inside house.

About water sources for domestic needs, when we considered wells and rainwater as unsafe sources of water, these showed no significant association with diarrhea (OR=1.6; 95% CI 0.99 – 2.7; p=0.06).

We also found no significant difference between storage containers without lid and storage containers with lid (OR=0.8; 95% CI 0.5-1.3; p=0.3).

4.3.5. Breastfeeding status of the child

There were 39 cases (19.5%) and 57 controls (14.25%) exclusively breastfed in the first six months of life. 161 cases (80.5%) and 343 controls (85.75%) were reported being breastfed not exclusively, in which there were 3 cases (1.5%) and 3 control (0.75%) not being breastfed at all. We found that there was no significant association between exclusive breastfeeding in the first 6 months of life and diarrhea among children recruited into the study (OR=0.7; 95% CI 0.4 – 1.1; p=0.1).

Table 4.5: Bivariate analysis of some risk factors associated with diarrhea among cases and controls

Potential risk factors	Cases *(n=200)*	Controls *(n=400)*	Matched odds ratio	95 % confidence interval	P value
Level of mother's education					
Primary	152 *(76%)*	238 *(59.5%)*	**2.2**	**1.5 – 3.2**	< 0.001
Primary	152	238	ref.		
Secondary	36	94	0.6	0.4 – 1.0	0.02
College/University	12	68	0.3	0.1 – 0.6	< 0.001
Marital status					
Single	4 *(2 %)*	4 *(1%)*	2.0	0.5 – 8.2	0.5
Married	196	391	0.5	0.1 – 2.0	0.5
Divorced	0	3			
Separated	0	2			
Mother's age under 26 (mean age)	81 (*40.5 %*)	183 (*45.75%*)	0.8	0.6 – 1.2	0.2
Mother's occupation as **Farmer**	154 *(77%)*	229 *(57.3%)*	**2.5**	1.7 – 3.7	**< 0.001**
Farmer	154	229	ref.		
Employed	33	81	0.6	0.4 – 1.0	0.03
Self-employed	13	90	0.2	0.1 – 0.4	< 0.001
Father's occupation as **Farmer**	119 *(60.4 %)*	131 *(33.5 %)*	**3.0**	2.1 – 4.3	**< 0.001**
Farmer	119	131	ref.		
Employed	39	101	0.4	0.3 – 0.7	< 0.001
Self-employed	39	159	0.3	0.2 – 0.4	< 0.001
Number of siblings of the child					
The child having sibling(s)	138 *(69%)*	216 *(54%)*	**1.9**	1.3 – 2.7	**< 0.001**
No sibling	62	184	ref.		
1 sibling	113	176	***1.9***	1.3 – 2.8	***< 0.001***
≥ 2 siblings	25	40	***1.9***	1.04 – 3.3	***< 0.001***
Poor economic status of the family	83 *(41.5 %)*	91 *(22.8 %)*	**2.4**	1.7 – 3.5	< 0.001
Room-sharing among more than 2 people	20 *(10%)*	23 *(5.8%)*	1.8	1.0 – 3.4	0.06

Potential risk factors	Cases *(n=200)*	Controls *(n=400)*	Matched odds ratio	95 % confidence interval	P value
Public latrine	4 *(2%)*	2 *(0.5%)*	4.1	0.6 – 32.2	0.1
Types of latrines used					
Old-type latrines	130 *(65%)*	197 *(49.3%)*	**1.9**	1.3 – 2.7	**< 0.001**
Modern toilet	70	203	ref.		
Two-compartment latrine	6	87	0.2	0.1 – 0.5	< 0.001
One-compartment latrine	124	110	3.3	2.2 – 4.8	< 0.001
Latrine cleaning					
1-2 times per week	72 *(36%)*	61 *(15.3%)*	**3.1**	2.1 – 4.7	**< 0.001**
1-2 times per week	72	61	ref.		
Everyday	60	136	0.4	0.2 – 0.6	< 0.001
Every time it is spoiled	68	203	0.3	0.2 – 0.5	< 0.001
Latrine-sharing among more than 5 people	28 *(14%)*	26 *(6.5%)*	**2.3**	1.3 – 4.1	**0.002**
Disposal of the child's stool	*(n=199)*	*(n=365)*			
Throw away in open surrounding	10 *(5%)*	5 *(1.4%)*	1.3	0.5 – 3.4	0.6
Throw away in open surrounding	10	5	ref.		
Buried	7	11	0.3	0.1 – 1.6	0.1
Put in the latrine	182	349	0.3	0.1 – 0.8	< 0.01
Disposal of waste water					
Pour away in open surrounding	31 *(15.5%)*	113 *(28.3%)*	0.5	0.3 – 0.7	0.001
Sewage system	169	287	ref.		
Pond	21	51	0.7	0.4 – 1.2	0.2
Garden	10	62	0.3	0.1 – 0.6	< 0.001
Using stool as fertilizer	13 *(6.5%)*	7 *(1.8%)*	**3.9**	1.5 – 9.9	**0.002**
No hand-washing for the child before eating	95 *(47.5%)*	148 *(37%)*	**1.5**	1.1 – 2.2	**0.014**
Hand-washing by mothers after going to toilet	52 *(26%)*	22 *(5.5%)*	**6.0**	3.5 – 10.3	**< 0.001**
Sometimes	148	378			
Usually					

Potential risk factors	Cases *(n=200)*	Controls *(n=400)*	Matched odds ratio	95 % confidence interval	P value
Hand-washing by mothers after helping children defecate					
Sometimes	91 *(45.5%)*	106 *(26.5%)*	**2.3**	1.6 – 3.3	**< 0.001**
Usually	109	294			
Hand-washing by mothers before feeding children					
Never	24 *(12%)*	7 *(1.8%)*	**7.7**	3.2 – 18.1	**< 0.001**
Never	24	7	ref.		
Sometimes	103	168	0.2	0.1 – 0.4	< 0.001
Usually	73	225	0.1	0.04 – 0.23	0.001
Hand-washing by mothers before preparing food for children					
Never	31 *(15.5%)*	14 *(3.5%)*	**5.1**	2.6 – 9.8	**< 0.001**
Never	31	14	ref.		
Sometimes	105	246	0.2	0.1 – 0.4	0.001
Usually	64	140	0.2	0.1 – 0.4	0.001
Hand-washing by mothers with					
Water only	161 *(80.5%)*	235 *(58.8%)*	**2.9**	1.9 – 4.3	**< 0.001**
Water and soap	39	165			
Methods of storing food for later use					
Unsafe methods	103 *(51.5%)*	90 *(22.5%)*	**3.7**	2.5 – 5.3	**< 0.001**
Not storing food for later use	59	206	ref.		
Storing food in refrigerator	38	104	1.3	0.8 – 2.1	0.3
Storing food in larder	71	47	**5.3**	3.2 – 8.7	**< 0.001**
Storing food in disk-cover	32	43	**2.6**	1.5 – 4.6	**< 0.001**
Not heating foods before reuse	1 *(3.1%)*	5 *(11.6%)*	0.2	0.03 – 2.2	0.23
Buying food for children from street vendor	43 *(21.5%)*	58 *(14.5%)*	**1.6**	1.04 – 2.5	**0.03**

Potential risk factors	Cases *(n=200)*	Controls *(n=400)*	Matched odds ratio	95 % confidence interval	P value
Kitchen cleaning					
Not regularly	113 *(56.5%)*	75 *(18.8%)*	5.6	3.9 – 8.2	**< 0.001**
Everyday	87	325	ref.		
2-3 times per week	70	70	**3.7**	2.5 – 5.6	**< 0.001**
Every week	43	5	**32.1**	12.4 – 83.5	**< 0.001**
Flies in the kitchen	117 *(58.5%)*	252 *(63%)*	0.8	0.6 – 1.2	0.3
Animals entering the kitchen	70 *(35%)*	123 *(30.8%)*	1.2	0.8 – 1.8	0.3
Keeping animals in kitchen overnight	8 *(4%)*	19 *(4.8%)*	0.8	0.3 – 2.1	0.7
Getting water from					
Unsafe water	177 *(88.5%)*	330 *(82.5%)*	1.6	0.99 – 2.7	0.06
Running water	23	70	ref.		
Well	177	326	1.7	0.99 – 2.7	0.05
Rainwater	0	4			
Storage container without lid	26 *(13%)*	64 *(16%)*	0.8	0.5 – 1.3	0.3
Storage container with lid	174	336			
Infrequent cleaning/emptying of storage container before refilling it with fresh water	81 *(40.5%)*	57 *(14.3%)*	**4.1**	2.8 – 6.1	< 0.001
Breastfeeding status of the child in the first 6 months of life					
Not exclusive	161 *(80.5%)*	343 *(85.75%)*	0.7	0.4 – 1.1	0.1
Exclusive	39	57	ref.		
Mixed	158	340	0.7	0.4 – 1.1	0.09
Not at all	3	3	1.5	0.2 – 9.7	0.7

4.4. Multivariate analysis

To identify risk factors that were independently associated with diarrhea and to control confounders, we put risk factors found significant in bivariate analysis into conditional logistic regression model [78].

Before being put into the model, the variables were converted to dichotomous variables.

Level of mothers' education was converted to a dichotomous variable that had 2 values namely primary and higher education. Primary education, which was considered low-level education, was used as the only variable in the model in order to find out whether (or not) an independently significant association existed between the mothers' low educational level and diarrhea.

Likewise, other variables were recoded in the same way. The converted-variables that were put into the model are shown in table 4.7.

In multivariate analysis, we found that some factors remained independently significant to the risk of diarrhea, including the child having sibling(s) (OR=1.9; 95% CI 1.2 – 3.2; p=0.01); irregular latrine cleaning (OR=4.4; 95% CI 2.4 – 8.1; $p<0.001$); latrine-sharing among more than 5 people (OR=2.8; 95% CI 1.3 – 6.2; p=0.008); irregular hand-washing by mothers after going to toilet(OR=4.5; 95% CI 2.1 – 9.5; $p<0.001$); No hand-washing by mothers before feeding children (OR=9.4; 95% CI 2.3 – 37.6; p=0.002); unsafe storage of food for later use (OR=3.4; 95% CI 2.0 – 5.7; $p<0.001$); irregular kitchen cleaning(OR=4.3; 95% CI 2.5 – 7.4; $p<0.001$); and infrequent cleaning/emptying of storage container before refilling it with fresh water (OR=7.7; 95% CI 4.4 – 13.5; $p<0.001$);

Results of multivariate analysis are illustrated in table 4.7.

Table 4.7: Multivariate analysis of risk factors associated with diarrhea

Risk factors	Sig.	Exp(B)	95% CI for EXP(B)	
			Lower	Upper
Mother's primary educational level	0.23	0.7	0.4	1.3
Mother's occupation as farmer	0.5	1.3	0.6	2.5
Father's occupation as farmer	0.85	0.9	0.5	1.7
Low economic status	0.59	0.7	0.2	2.5
The child having siblings	**0.01**	**1.9**	**1.2**	**3.2**
Old-type latrines	0.13	0.6	0.3	1.2
Irregular latrine cleaning	**0.001**	**4.4**	**2.4**	**8.1**
Latrine-sharing among more than 5 people	**0.008**	**2.8**	**1.3**	**6.2**
Using stool as fertilizer	0.25	2.1	0.6	7.1
No hand-washing for the child before eating	0.24	1.4	0.8	2.3
Irregular hand-washing by mothers after going to toilet	**0.001**	**4.5**	**2.1**	**9.5**
Irregular hand washing by mothers after helping children to defecate	0.27	1.4	0.8	2.5
No hand-washing by mothers before feeding children	**0.002**	**9.4**	**2.3**	**37.6**
No hand-washing by mothers before preparing foods for children	0.51	1.5	0.5	4.6
Hand-washing by mothers with water only	0.07	1.7	0.96	3.0
Unsafe storage of food for later use	**0.001**	**3.4**	**2.0**	**5.7**
Buying foods for children from street vendors	0.46	1.3	0.7	2.3
Irregular kitchen cleaning	**0.001**	**4.3**	**2.5**	**7.4**
Infrequent cleaning/emptying of storage container before refilling it with fresh water	**0.001**	**7.7**	**4.4**	**13.5**

4.5. Laboratory results

Among 200 stool samples collected in the study, we detected 109 samples (54.5%) positive to pathogens causing diarrhea, in which entero pathogenic *Escherichia coli* (EPEC) was isolated from 54 samples (27%); *rotavirus* was detected in 50 samples (25%); *Shigella* in 8 samples (4%) and *Entamoeba hystolytica* (cyst only) in 23 samples (11.5%). We isolated 1 *Shigella flexneri* and 7 *Shigella sonnei*.

Furthermore, we identified co-infection in 27 cases (13.5%), including 13 cases (6.5%) infected to both *EPEC* and *rotavirus;* 5 cases to *EPEC* and *Entamoeba hystolytica* (2.5%); 6 cases to *Shigella* and *Entamoeba hystolytica* (3%)*;* 1 case to *Shigella* and *rotavirus* (0.5%); and 2 cases to *rotavirus* and *Entamoeba hystolytica* *(*1%). Frequency of pathogens identified in 200 stool samples is shown in table 4.8.

Table 4.8: Frequency of pathogens identified in 200 collected stool samples

Pathogens	**Cases *(N= 200)***	**Percentage**
E.coli (EPEC-entero pathogenic *E. coli*)	54	27
Shigella (*Sh. flexneri* and *Sh. sonnei*)	8	4
Entamoeba hystolytica (cyst)	23	11.5
Rotavirus	50	25
Vibrio cholera	0	0
Giardia lamblia	0	0
Cyclospora cayetanensis	0	0
Coinfection		
- *EPEC & E.hystolytica*	5	2.5
- *EPEC & rotavirus*	13	6.5
- *Shigella & E. hystolytica*	6	3
- *Shigella & rotavirus*	1	0.5
- *Rotavirus & E. hystolytica*	2	1
Total of co-infection cases	27	13.5
Total of cases positive to pathogens	**109**	54.5

In this study, we observed no distinct difference in number of cases detected positive to bacteria between rainy, hot months (summertime) and dry, cold months (wintertime). But number of rotavirus-identified-cases was much higher during winter than that during summer: 41 cases compared to 9 cases respectively. (*See table 4.9* below)

Table 4.9: Distribution of pathogen-identified- cases by month

	July	**Aug**	**Sep**	**Oct**	**Nov**	**1-15 Dec**	**Total**
EPEC	8	9	10	16	6	5	54
Shigella	3	0	0	1	3	1	8
Rota	7	2	0	17	16	8	50
E. hystolytica	8	7	0	2	4	2	23
Total	26	18	10	36	29	16	109

Because of the fact that we identified no pathogen in 91 stool samples (45.5%). We wanted to examine which factors that increased the risk of diarrhea caused by identified pathogens. We conducted statistical analyses of potential risk factors among 109 pathogen-identified-cases and 218 matched controls. Results of analysis are summarized in table 4.10.

As seen in this table, comparing to results of analysis among 200 cases and 400 controls, we found 2 different factors which remained independently significant to the risk of diarrhea with an identified pathogen, namely mothers' occupation as farmer (OR=2.7; 95% CI 1.1 – 7.0) and mothers washing hands with water only instead of by soap and water (OR=2.3; 95% CI 1.05 – 5.1). Other factors found to independently associate with positive microbiological diagnosis were the following: the child having sibling(s) (OR=2.8; 95% CI 1.4 – 5.5); irregular latrine cleaning (OR=5.0; 95% CI 2.2 – 11.2); irregular hand-washing by mothers after going to toilet(OR=3.3; 95% CI 1.2 – 9.0); unsafe storage of food for later use (OR=3.1; 95% CI 1.5 - 6.4); irregular kitchen cleaning(OR=3.3; 95% CI 1.6 – 6.9); and infrequent cleaning/emptying of storage container before refilling it with fresh water (OR=5.2; 95% CI 2.4 – 14.4).

Table 4.10: Bivariate and multivariate analyses of potential risk factors among 109 pathogen-positive-cases and 218 matched controls.

Potential risk factors	Case *N=109*	Control *N=218*	Crude OR (95%CI)	Adjusted OR (95% CI)
Mother's primary educational level	82	127	2.2 (1.3-3.6)	0.7 (0.3-1.6)
Mother's occupation as farmer	89	117	3.8 (2.2-6.7)	**2.7 (1.1-7.0)**
Father's occupation as farmer	59	74	2.4 (1.5-3.9)	0.7 (0.3-1.6)
Room-sharing among over 2 persons	13	10	2.8 (1.2-6.7)	1.1 (0.4-3.7)
Low economic status	48	45	3.0 (1.8-5.0)	3.1 (0.5-20.0)
The child having sibling(s)	79	119	2.2 (1.3-3.6)	**2.8 (1.4-5.5)**
Old-type latrines	73	106	2.1 (1.3-3.5)	0.7 (0.3-1.5)
Irregular latrine cleaning	41	38	2.9 (1.7-4.8)	**5.0 (2.2-11.2)**
Latrine-sharing among more than 5 people	15	15	2.2 (1.01-4.6)	1.9 (0.6-5.5)
Using stool as fertilizer	8	5	3.4 (1.1-10.6)	2.4 (0.5-10.8)
No hand-washing for the child before eating	51	75	1.7 (1.1-2.7)	1.3 (0.6-2.8)
Irregular hand-washing by mothers after going to toilet	27	11	6.2 (2.9-13.1)	**3.3 (1.2-9.0)**
Irregular hand washing by mothers after helping children to defecate	48	57	2.2 (1.4-3.6)	1.1 (0.5-2.4)
No hand-washing by mothers before feeding children	12	6	4.3 (1.6-12.0)	4.9 (0.9-27.2)
No hand-washing by mothers before preparing foods for children	18	10	4.1 (1.8-9.3)	1.6 (0.4-6.5)
Hand-washing by mothers with water only	92	130	3.7 (2.0-6.6)	**2.3 (1.05-5.1)**
Unsafe storage of food for later use	55	40	4.5 (2.7-7.5)	**3.1 (1.5-6.4)**
Buying foods for children from street vendors	22	38	1.2 (0.7-2.1)	1.2 (0.5-2.7)
Irregular kitchen cleaning	59	39	5.4 (3.2-9.0)	**3.3 (1.6-6.9)**
Infrequent cleaning/emptying of storage container before refilling it with fresh water	39	35	2.9 (1.7-5.0)	**5.2 (2.4-11.4)**

For pathogens identified at high percentages in the study, such as entero pathogenic *Escherichia coli* and *rotavirus*, we also conducted statistical analyses of risk factors among 54 EPEC-identified-cases and 108 matched controls; and among 50 *rotavirus*-identified cases and 100 matched controls.

According to results of the two analyses shown in table 4.11, there were a significant association between some risk factors and the occurrence of diarrhea caused by EPEC. Those factors were mother's farming occupation; the child having sibling(s); irregular latrine cleaning; mothers washing hands with water only; unsafe storage of food for later use; irregular kitchen cleaning; and infrequent cleaning/emptying of storage container before refilling it with fresh water.

However, irregular cleanings of latrine and kitchen are identified as the only two factors that increase the risk of diarrhea caused by *rotavirus*.

Table 4.11: Results of bivariate and multivariate analysis of risk factors associated with diarrhea caused by EPEC and Rota virus

Potential risk factors	*Entero pathogenic Escherichia coli*				*Rota virus*			
	Case *N=54*	**Control** *N=108*	**Crude OR (95%CI)**	**Adjusted OR (95% CI)**	**Case** *N=50*	**Control** *N=100*	**Crude OR (95%CI)**	**Adjusted OR (95% CI)**
Mother's primary educational level	42	62	2.6 (1.2-5.5)	0.6 (0.2-2.0)	38	58	2.3 (1.1-4.9)	0.4 (0.1-1.6)
Mother's occupation as farmer	48	58	6.9 (2.7-17.5)	**5.1 (1.04-25.4)**	43	53	5.4 (2.2-13.3)	2.4 (0.5-11.5)
Father's occupation as farmer	29	37	2.3 (1.2-4.4)	0.9 (0.3-2.6)	32	32	4.0 (1.9-8.2)	3.0 (0.8-10.9)
The child having sibling(s)	38	56	2.2 (1.1-4.4)	**3.7 (1.2-11.0)**	38	12	2.3 (1.1-5.1)	1.9 (0.6-6.2)
Low economic status	21	27	1.9 (0.95-3.8)	1.5 (0.5-4.6)	28	22	4.5 (2.2-9.4)	1.6 (0.4-5.7)
Old-type latrines	35	54	1.8 (0.9-3.6)	0.7 (0.2-2.5)	36	47	2.9 (1.4-6.0)	0.7 (0.2-3.3)
Irregular latrine cleaning	16	18	2.1 (0.97-4.6)	**5.1 (1.3-20.4)**	22	15	4.5 (2.0-9.7)	**6.1 (1.5-24.8)**
No hand-washing for the child before eating	29	35	2.4 (1.2-4.7)	2.6 (0.9-7.8)	27	31	2.6 (1.3-5.3)	2.5 (0.7-8.9)
Mother sometimes washing hands after going to toilet	13	6	5.4 (1.9-15.1)	3.5 (0.8-14.8)	14	4	9.3 (2.9-30.2)	4.7 (0.8-29.0)
Mother sometimes washing hands after helping the child defecate	23	28	2.1 (1.1-4.2)	1.2 (0.4-3.6)	21	29	1.8 (0.9-3.6)	0.4 (0.1-1.9)
No hand-washing by mothers before preparing food for children	8	2	9.2 (1.9-45.1)	6.3 (0.7-53.3)	8	5	3.6 (1.1-11.7)	5.8 (0.9-38.5)
Washing mother's hands with water only	47	68	4.0 (1.6-9.6)	**3.9 (1.1-14.5)**	41	60	3.0 (1.3-6.9)	1.2 (0.4-4.2)
Unsafe storage of food for later use	31	23	5.0 (2.5-10.1)	**3.8 (1.4-10.3)**	26	19	4.6 (2.2-9.7)	3.2 (0.9-10.8)
Irregular kitchen cleaning	28	19	5.0 (2.4-10.4)	**3.5 (1.2-10.5)**	37	11	23 (9.5-56.1)	**10.3 (3.0-35.5)**
infrequent cleaning/emptying of storage container before refilling it with fresh water	22	13	5.0 (2.2-11.1)	**9.9 (3.0-32.7)**	19	20	2.5 (1.2-5.2)	2.7 (0.8-9.6)

CHAPTER 5

DISCUSSION

It is widely recognized that diarrhea is a major cause of morbidity and mortality among children, especially children in developing countries.

Vietnam is a low-income country, where diarrhea is the second leading cause of deaths among children less than five years of age. Low socio-economic status, limited education, poor environmental sanitation and low hygienic practices pose a serious threat to people's health, especially children's health.

Risk factors for diarrhea vary with the child's age, the pathogens involved, and the local environment. My study is an addition to few studies that have been conducted so far in rural areas in Vietnam.

Dong Anh District was chosen for the study because it has the characteristics of rural areas where clean water, sanitation and hygiene remain problems. So far, no similar research, identifying the most common causes of diarrhea and risk factors associated with diarrhea among children less than five, has been conducted in the district.

5.1. Strengths of the study

In a hospital-based study, we could easily select consecutive subjects who fulfilled the inclusion criteria. This also helped us to avoid selection bias by rejecting subjects who had conditions listed in the exclusion criteria. Moreover, in the context of limited time and money, a hospital-based study was relatively easy and inexpensive to conduct.

According to C. H. Hennekens *et al* [79], age and sex are associated with virtually all diseases and are related to the presence or level of many exposures. In our study, sex and age group were used as matching criteria to select controls. The matching allowed us to control two potential confounders, namely sex and age.

As discussed section in 3.4.2, selection of hospitalized controls helped us to reduce the potential for recall bias, and to minimize bias due to non-response.

Trainings for research assistants and pre-testing conducted before data collection ensured a standardized way of colleting information. Among research assistants who work in Dong Anh Hospital, there were two medical doctors and one nurse. They had experience in clinical medicine and in doing research. That also ensured quality of collected data as well as recruitment of subjects that conformed to inclusion and exclusion criteria.

Frequent communication between the principal researcher and the assistants helped to deal with some problems that arose in the process of collecting data.

Vietnamese language, which is the sole language for communication in the area, was used for interview. That reduced misunderstanding between interviewers and mothers.

5.2. Limitations of the study

Selection of hospitalized controls led to a limitation that the experience of the controls may not accurately represent the exposure distribution in the population from which the cases derived.

Using sex and age as matching criteria did not allow evaluation of potential effect of sex and age on risk of diarrhea. Matching may also have introduced bias because controls were no longer representative of source population from which the cases derived.

Due to lack of resources, we could not detect *Campylobacter jejuni* which had been identified to be one of the most common causes of diarrhea in previous studies[71]. Also, detection of other diarrheagenic *E. coli* strains by PCR technique, like EAggEC, ETEC, and EIEC that were found to be commonly causing diarrhea among children under five of age in Hanoi [73], was impossible in context of the limited budget.

We should have come to subjects' houses to carry out observation in sanitation, hygiene and water – related – practices. However, it was impossible in context of limited time and manpower. Moreover, some questions may have been so sensitive that some interviewees did not feel confident to give an honest answer. The above-mentioned limitations may result in information bias.

Moreover, it is well known that unhygienic practices regarding sanitation, food hygiene and drinking water are potential risk factors associated with diarrhea, so some interviewers might produce bias (interviewer bias) by inflating exposure information in cases group.

Another limitation is that some questions, especially questions in the section on knowledge of diarrhea, were vague. Mothers' answers were sometimes very difficult to interpret. The categorization of knowledge of diarrhea was arbitrarily chosen, resulting in invalid evaluation.

We realized that those variables were not interpretable. Therefore we decided not to put them in bivariate and multivariate analysis.

Lastly, when a variable was dichotomized one that was put in the conditional logistic regression model, some information of this variable was lost. Moreover, putting many independent variables in the model might lead to less precise results and increase the risk of doing “false positive” findings.

5.3. The results of the study

5.3.1. Seasonal distribution

In figure 4.1, we observed a decrease in number of cases recruited in September (20 cases) and December (14 cases) compared to other months. The reason why only 14 cases were recruited in December was simply that we finished selecting cases in the first half of the month when reaching the total of 200 cases. Transitional period between hot, rainy season and cold, dry season may result in the lower number of cases recruited in September.

In northern Vietnam, the rainy and hot season lasts from May to September. The hottest weather occurs from July to August. Hot weather with high humidity is good conditions for bacteria to grow. That easily leads to contamination of food. In rural areas, due to low income, many people cannot afford to buy a refrigerator for food storage. Moreover, unhygienic environmental conditions with presence of cockroaches and flies also increase the risk of diarrhea. According to data reported by NIHE [70], a higher prevalence of diarrhea has been observed in the rainy season in northern Vietnam. Also, some studies conducted in other country found that the number of patients with diarrhea increased with the onset of the monsoon rains and peaked during the months of maximum rainfall [8, 42, 44].

While bacteria are the most common causes of diarrhea among children in the summertime, *rotavirus* is the leading cause of diarrhea among children in the wintertime [8, 69, 80, 81]. Some studies conducted in Vietnam indicated that *rotavirus* infection among children occurred all year round, but the prevalence was higher from September to December in northern Vietnam. During the other months of the year, the number of infected cases decreased [69, 80]. Likewise, in our study, of 50 *rotavirus*-positive cases, 41 cases (82%) occurred from October to December, 2005.

5.3.2. Age distribution

According to Molbak K *et al* [13] and Woldemicael G [17], the rates of diarrhea were highest for children 6-11 months of age, remained at a high level among the 1-year-old children and decreased when children got older.

We observed that cases were mostly children less than 24 months of age (*Figure 4.2*), in which there were 90 cases aged less than 12 months, making up the highest rate (45 % of the total). Number of cases decreased in older children, 79 cases in children aged 12 - 23 months and 30 cases in children aged 24 - 36 months. There was only a case aged 48 months. A decrease in number of cases among older children might be resulted from a fact that the immune system in older children got stronger in resisting against agents.

Symptoms of diarrhea in older children may be lighter compared to younger children. Therefore they could be sent to local medical clinics or commune medical stations for treatment instead of Dong Anh Hospital.

5.3.3. Sex distribution

The reason for the sex difference regarding rate of diarrhea is far from clear. Gender preference is an unlikely explanation because the difference has been found in very different cultures and in studies with different methodological approaches.

Among the total 200 cases recruited into the study, the number of boys was higher girls in nearly all age groups (*Figure 4.2*).

A biological explanation may be related to the fact that boys during infancy have to build a larger muscle mass than girls. Consequently, boys might have increased demands for micronutrients, and are therefore more at risk of a negative balance, including lack of vitamin A and zinc. This vulnerability might increase the risk of diarrhea, and place the boys as the weaker sex regarding infections [13].

Among older children, because boys are more active than girls, boys tend to move around and touch objects in the surrounding ground, whereas girls might tend to stay close to their mothers and/or to play with more hygienic toys.

5.3.4. Knowledge of diarrhea in mothers

Although it is a suburban district of Hanoi, Dong Anh remains a poor rural area where 70 % of the population live from farming. GDP per capita was 200 USD in 2003. As a result of a low level of education, public perception on diarrhea prevention is still a problem. Moreover, due to hard farming occupation, many people usually do not have time to access information regarding diarrhea prevention.

Among 600 mothers, 279 and 280 mothers had acquired most of their knowledge of diarrhea through television and village health workers respectively, meanwhile 209 had their knowledge mainly from medical facilities. Only 101 mothers had learnt about diarrhea through reading and 78 through radio while 63 had their knowledge through interactions with their friends and other people. This shows that television, village health workers and medical facilities are the main sources of information on diarrhea to the public.

Due to low economic status, television is considered an expensive item that many families cannot afford. In addition, because of limited budget, activities to improve public perception could not be regularly held in the community. Low incentives to village health workers might reduce their enthusiasm. Without village health workers, local medical clinics and commune medical stations could hardly provide enough health information to the population.

Consequently, among the 600 mothers who were recruited into the study, as many as 20 mothers in both groups did not have any knowledge of diarrhea. 63 mothers had poor knowledge of how diarrhea spread, while 34 mothers had poor knowledge of diarrhea prevention (*table 4.4*). In nearly all categories, mothers in the control group had better knowledge of diarrhea compared with mothers in the case group. The difference between 2 groups was statistically significant.

Although criteria for the categorization were arbitrarily chosen and sometimes led to inaccurate evaluation, we should consider mothers' knowledge as a very important factor that reduces the risk of diarrhea.

5.3.5. Risk factors associated with diarrhea

Multivariate analysis is used as a standard method to identify risk factors associated with a disease while adjusting for potential confounders [78]. In this study, using conditional logistic regression model, we found 8 factors independently associated with the risk of diarrhea, including the child having sibling(s); irregular latrine cleaning; Latrine-sharing among more than 5 people; irregular hand washing by mothers after going to toilet; no hand-washing by mothers before feeding children; unsafe storage of food for later use; irregular kitchen cleaning and infrequent cleaning/emptying of storage container before refilling it with fresh water (*table 4.7*).

The child having sibling(s)

In Vietnam, each married couple is allowed to have at most 2 children. Due to low public awareness of birth-control, many families have more 2 children, especially in rural areas. Another explanation for failure in family planning is that people prefer a son to a daughter in order to maintain the family line. As a result of that old thinking, child-bearing will continue until the family has a boy child.

In our study, there were 25 children (12.5%) in the case group having 2 siblings. In control group, number of children having 2 siblings was 37 (9.25%). Only 3 children (0.75%) have 3 siblings.

When we categorized number of siblings of children into 2 group: having sibling(s) or not, we found that the risk of having diarrhea among children who have one or more siblings is nearly 2 times higher than those who are only child (OR=1.9; 95 % CI 1.2 – 3.2; p=0.01).

People have to spend much time earning for their living, therefore, they do not have enough time to take care of their children. This is especially true of people in the rural areas whose income is low and very labor-intensive. Along with unhygienic environmental situation in rural areas, lack of parents' care of children leads to a higher risk of diarrhea among children who have siblings than among those who are the only child.

Differently from the results of J. Gascon's study [28] that indicated birth order was associated with risk of diarrhea, with latest born having a lower risk of diarrhea, there was no significant association between birth order and diarrhea in our study.

Irregular latrine cleaning and ***latrine-sharing among more than 5 people***

Some studies [24, 25, 29] found that unhygienic latrines and sharing latrines increased the risk of diarrhea. In rural areas, newspapers, old notebook papers or other low-quality toilet papers are used after going to toilet in many families. Those papers cannot be flushed with water but they are usually kept in a bucket placed in the latrine. In this situation, cleaning the latrine less than 3 times per week was considered irregular. Also, the number of 5 people was used as cut-off point to categorize the number of people sharing a latrine into 2 values: more than 5 and 5 downwards. Obviously, more people sharing a latrine reduce the hygienic level of the latrine. The findings that irregular latrine cleaning and latrine-sharing among many people are associated with the risk of diarrhea are compatible with the results indicated in a large number of studies so far.

Irregular hand washing by mothers after going to toilet and ***no hand-washing by mothers before feeding children***

Likewise, washing hands have been clearly shown to reduce the occurrence of diarrhea [82, 83].

In Vietnam, mothers spend much time raising children and doing housework while fathers are responsible for making money. In rural areas mothers are also bread winners in the families. Mothers actually are more strenuous compared with fathers. Their tiredness might be a reason for not paying attention to hand-washing. In addition, low educational level as well poor knowledge of diarrhea also explain why many mothers do not realize that their sanitation and food hygiene-related-practices are very important to children's health.

In the study the risk of diarrhea among children whose mothers had the two poor hand washing-related-practices was 4.5 times and 9.4 times higher than that among those whose mothers paid attention to washing their hand after going to toilet and before feeding children respectively.

Irregular kitchen cleaning

In rural areas, very few families can afford cooking with electric or gas cookers. Families often use wood, coal, straw, or even fallen leaves for cooking. Storage of these materials in the kitchen facilitates the development of diarrhea transmitting vectors, such as flies and cockroaches. Furthermore, when people burn these materials for cooking, they have to touch the materials. This is likely to lead to contamination of food.

In addition, when the kitchens are built close to livestock stables or poultry feeding place, it might cause an unhygienic situation of kitchen.

Based on these realities, cleaning the kitchen 3 times or less per week was considered irregular. The risk of diarrhea among children whose parents cleaned kitchen irregularly was 4.3 times higher than among those whose parents cleaned kitchen every day.

Unsafe storage of food for later use

In Dong Anh District, a fridge is a luxury item in a family. Only well-off families can afford a fridge. In addition, low demands by families of food storage and the high cost of electricity make the fridge impractical home equipment.

In fact, storing food in larder and disk-cover is very popular in the area. Under hot weather conditions and without refrigeration, food is easily contaminated by pathogens. Besides, larder or disk-cover merely prevents flies but not cockroaches that are important vectors to transmit pathogens.

Storing food in larder and disk-cover were considered unsafe methods and were found significant to increase the risk of diarrhea (OR=4.3; $p < 0.001$).

Infrequent cleaning/emptying of container before refilling it with fresh water

Peter Kjær Jensen *et al* [4, 9] found that transmission of diarrhea occurs easily when in-house water storage facilities or/and water sources are contaminated (corresponding to domestic domain and public domain contamination). Most of transmission of diarrhea occurs in the domestic domain.

In the district, running water is not available in villages, except Dong Anh Town. People mainly use water from wells for drinking and other domestic needs. Water is usually stored in concrete tanks and big jars. Concrete tanks are rarely cleaned and emptied more than once or twice in a year before being refilled with fresh water. Many people do not wash their hands before getting water from storage containers. Thus contamination easily occurs in those containers.

All families boiled water before drinking. However they used the same source of water to wash utensils for feeding children. This washing could result in contaminated utensils, indirectly causing diarrhea in children.

5.3.6. Laboratory results

As we already explained, we had to leave out tests for *Campylobacter jejuni* and diarrheagenic *E. coli* other than EPEC in this study. In addition, some patients were treated by oral antibiotics before admission. Owing to logistic problems, some stool samples were transported to NIHE as late as 3-5 days after being collected. These problems contributed to the fact that we detected no pathogens in 91 stool samples.

For financial reasons, we could only identify EPEC by polyvalent antiserum. We could not afford to have monovalent antiserum for identification of serogroups as well as serotypes of EPEC strains isolated in the study.

Among pathogens identified in 200 stool samples, *E. coli* made up the highest number (54 cases equivalent to 27%), *rotavirus* 50 (25%) and *Shigella* 8 (4%). For Shigella serogroups, we identified 7 samples positive to *Shigella sonnei* against only one sample positive to *Shigella flexneri.*

Entamoeba hystolytica was detected in 23 samples but we did not consider this pathogen as the direct cause of diarrhea because it was identified in its inactivated form (cyst).

According to Nguyen Vu Trung *et al* [80], *rotavirus* infection was detected in association with either diarrheagenic *E. coli* or *Shigella*. The most frequent combinations were *rotavirus-EAggEC* and *rotavirus-EPEC*.

In this study, we detected 13 cases which were co-infected with *rotavirus* and *EPEC* and one case to *rotavirus* and *Shigella sonnei.*

In 200 samples, we detected no sample positive to *Vibrio cholera, Samonella, Giardia lamblia* and *Cyclospora cayetanensis.*

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSION

The results of the study show that the factors, namely the child having sibling(s), irregular latrine cleaning; Latrine-sharing among more than 5 people; irregular hand washing by mothers after going to toilet; no hand-washing by mothers before feeding children; unsafe storage of food for later use; irregular kitchen cleaning and infrequent cleaning/emptying of storage container before refilling it with fresh water, were significantly associated with the risk of diarrhea among children less than five of age admitted to Dong Anh Hospital.

Entero pathogenic *Escherichia coli* (EPEC), *rotavirus* and *Shigella spp.* are found to be common pathogens causing diarrhea among hospitalized children.

6.2. RECOMMENDATIONS

- Encourage mothers to wash their hands with soap before feeding children or after going to toilet.
- Arrange cooking place and food-storing place in the kitchen as separately as possible.
- Advise people to store materials for cooking in a separate place instead of in kitchen.
- Recommend people to buy food daily and cook for every meal.

We also recommend other methods that could effectively prevent diarrhea, as follows:

- Mobilize all resources in the community to build kindergartens in all villages if possible.
- Implement IMCI to control diarrhea. This strategy includes following interventions: ORT, continuation of feeding during diarrhea, intensive care for severe dehydration, selective antibiotic therapy, and seeking medical care when needed.

ANNEX 1

REFERENCES

1. Urio EM, Collison EK, Gashe BA, Sebunya TK and Mpuchane S. Shigella and Salmonella strains isolated from children under 5 years in Gaborone, Botswana, and their antibiotic susceptibility patterns. Trop Med Int Health; 2001; 6(1): 55–9.

2. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organ 2003; 81 (3): 197-204.

3. Vesikari T and Torun B. Diarrheal Diseases. In: Kari SL, Staffan B, Makela PH, Miikka P, editors. Health and Disease in developing countries. Macmillan Education Ltd. London and Oxford, 1994. p.136-46.

4. Jensen PK, Jayasinghe G, van der Hoek W, Cairncross S, Dalsgaard A. Is there an association between bacteriological drinking water quality and childhood diarrhea in developing countries? Trop Med Int Health**;** 2004; 9 (11): 1210–15.

5. Armon K, Stephenson T, MacFaul R, Eccleston P, Werneke U. An evidence and consensus based guideline for acute diarrhoea management. Arch Dis Child; 2001; 85**:**132-42.

6. Warren PB. Diarrhea in Childhood. Department of Pediatrics. The University of Iowa, USA. 5/2003.

7. Gracey M. Diarrhoeal disease and malnutrition. Churchill Livingstone, Edinburgh, 1985.

8. Gracey M. Diarrhea and Malnutrition: A Challenge for Pediatricians. J Pediatr Gastroenterol Nutr; 1996, 22(1): 6-16.

9. Jensen PK, Ensink JH, Jayasinghe G, van der Hoek W, Cairncross S, Dalsgaard A. Domestic transmission routes of pathogens: the problem of in-house contamination of drinking water during storage in developing countries. Trop Med Int Health; 2002; 7(7): 604–9.

10. Curtis V, Cairncross S, Yonli R. Review: Domestic hygiene and diarrhoea – pinpointing the problem. Trop Med Int Health; 2000; 5 (1): 22–3.

11. Jane SH, Swanson LN. Diarrhea. A Jobson Publication.
Available from URL: http://www.uspharmacist.com
12. Banerjee B, Hazra S, Bandyopadhyay D. Diarrhea Management Among Under Fives. Institute of Hygiene and Public Health, India, 2003.
Available from URL: http://www.indianpediatrics.net/mar2004
13. Molbak K. The epidemiology of diarrheal diseases in early childhood: A review of community studies in Guinea-Bissau. University of Copenhaghen, 2000.
14. Abram SB, editor. Control of Communicable diseases Manual. 16th ed. Am Public Health Assoc. 1995: 250-4.
15. Waldman R, Fontaine O, Richard L. Epidemic dysentery. A supplement to Dialogue on Diarrhoea. Published by AHRTAG, UK.1994.
16. WHO. Department of Child and Adolescent Health and Development. The evolution of diarrheal and acute respiratory diseases control at WHO. World Health Organ. 1999.
17. Woldemicael G. Diarrheal morbidity among children in Eritrea: environmental and socio-economic determinants. J Health Popul Nutr; 2001; 19 (2): 83-90.
18. Yassin K. Morbidity and risk factors of diarrheal disease among under-five children in rural upper Egypt. J Trop Pediatr; 2000; 46 (5): 282-7.
19. Teklemariam S, Getaneh T, Bekele F**.** Environmental determinants of diarrheal morbidity in under-five children, Keffa-Sheka zone, south west Ethiopia. Ethiop Med J; 2000; 38(1):27-34.
20. Karim AS, Akhter S, Rahman MA, Nazir MF. Risk factors of persistent diarrhea in children below five years of age. Indian J Gastroenterol. 2001; 20(2):59-61.
21. Jinadu MK, Olusi SO, Agun JI, Fabiyi AK. Childhood diarrhoea in rural Nigeria. I. Studies on prevalence, mortality and socio-environmental factors. J Diarrhoeal Dis Res. 1991; 9(4):323-7.
22. Marjatta BS. Water supply and diarrhea in East African community. A case-control study on the quality of water supplies and the occurrence of diarrhea among small children in a rural area of Western Kenya. University of Oulu Printing Center, 1994: 37-57.

23. Molbak K, Jensen H, Ingholt L, Aaby P. Risk factors for diarrheal disease incidence in early childhood: a community cohort study from Guinea-Bissau. Am J Epidemiol. 1997; 146(3):273-82.
24. Etiler N, Velipasaoglu S, Aktekin M. Risk factors for overall and persistent diarrhea in infancy in Antalya, Turkey. Public Health, 2004; 118 (1): 62-9.
25. Wijewardene K, Fonseka P, Wijayasiri WA. Risk factors contributing to acute diarrhoeal disease in children below five years. Ceylon Med J, 1992; 37(4):116-9.
26. Tumwine JK, Thompson J, Katua-Katua M, Mujwajuzi M, Johnstone N, Porras I. Diarrhoea and effects of different water sources, sanitation and hygiene behaviour in East Africa. Trop Med Int Health. 2002; 7(9):750-6.
27. Sobel J, Gomes TA, Ramos RT, Hoekstra M, Rodrigue D, Rassi V, Griffin PM. Pathogen-Specific Risk Factors and Protective Factors for Acute Diarrheal Illness in Children Aged 12–59 Months in Sao Paulo, Brazil. Clin Infect Dis. 2004; 38 (11): 1545-51.
28. Gascon J, Vargas M, Schellenberg D, Urassa H, Casals C, Kahigva E, Aponte JJ, Mshinda H, Vila J. Diarrhea in Children under 5 Years of Age from Ifakara, Tanzania. J Clin Microbiol. 2000; 38 (12): 4459–62.
29. Brooks JT et al. Epidemiology of sporadic bloody diarrhea in rural western Kenya. Am J Trop Med Hyg. 2003; 68 (6): 671–7.
30. Ghosh S, Sengupta PG, Mondal SK, Banu MK, Gupta DN, Sircar BK. Risk behavioural practices of rural mothers as determinants of childhood diarrhoea. J Commun Dis. 1997; 29(1):7-14.
31. Aulia H, Surapaty SC, Bahar E, Susanto TA, Roisuddin, Hamzah M, Ismail R. Personal and domestic hygiene and its relationship to the incidence of diarrhoea in south Sumatera. J Diarrhoeal Dis Res. 1994; 12(1):42-8.
32. Alam N, Wojtyniak B, Henry FJ, Rahaman MM. Mothers' personal and domestic hygiene and diarrhoea incidence in young children in rural Bangladesh. Int J Epidemiol. 1989; 18(1):242-7.
33. Gorter AC, Sandiford P, Pauw J, Morales P, Perez RM, Alberts H. Hygiene behaviour in rural Nicaragua in relation to diarrhoea. Int J Epidemiol. 1998; 27(6):1090-100.

34. Curtis V, Cairncross S, Yonli R. Review: Domestic hygiene and diarrhoea – pinpointing the problem. Trop Med Int Health; 2000; 5 (1): 26–30.
35. Long K, Vasquez-Garibay E, Mathewson J, de la Cabada J, DuPont H. The impact of infant feeding patterns on infection and diarrheal disease due to enterotoxigenic *Escherichia Coli*. Salud Publica Mex. 1999; 41(4):263-70.
36. VanDerslice J, Popkin B, Briscoe J. Drinking-water quality, sanitation, and breast-feeding: their interactive effects on infant health. Bull World Health Organ. 1994; 72(4):589-601.
37. Ahiadeke C. Breast-feeding, diarrhoea and sanitation as components of infant and child health: a study of large scale survey data from Ghana and Nigeria. J Biosoc Sci. 2000; 32(1):47-61.
38. Public Health News Center. Johns Hopkins University. Exclusive Breastfeeding Training For Mothers Helps Reduce Diarrheal Disease Among Infants. The University. 2005.
39. Brown KH. Diarrhea and Malnutrition. J Nutr. 2003; 133 (1): 328S-332S.
40. Rice AL, Sacco L, Hyder A, Black RE. Malnutrition as an underlying cause of childhood deaths associated with infectious diseases in developing countries. Bull World Health Organ. 2000; 78(10):1207-21.
41. National Medical Society, USA. Diarrhea in HIV-Infected Patients.
Available from URL: http://www.medical-library.org/
42. Adkins HJ, Escamilla J, Santiago LT, Ranoa C, Echeverria P, Cross JH. Two-year survey of etiologic agents of diarrheal disease at San Lazaro Hospital, Manila, Republic of the Philippines. J Clin Microbiol. 1987; 25(7): 1143–7.
43. Henry FS, Patwary Y, Huttly SR, Aziz KM. Bacterial contamination of weaning foods and drinking water in rural Bangladesh. Epidemiol Infect. 1990; 104(1):79-85.
44. Pinfold JV, Horan NJ, Mara DD. Seasonal effects on the reported incidence of acute diarrhoeal disease in northeast Thailand. Int J Epidemiol. 1991; 20(3):777-86.
45. Teshima A et al. Climate impact on seasonal patterns of diarrhea diseases in Tropical area. University of Kyoto, Japan, 2004.
46. Salazar-Lindo E, Pinell-Salles P, Maruy A, Chea-Woo E. El Nino and diarrhoea and dehydration in Lima, Peru. Lancet. 1997; 350 (9091):1597-8.

47. Protection of the Human Environment - Task Force on Climate and Health. El Nino and Health. WHO, Geneva, 1999, 22-23.
48. Pascual M, Rodo X, Ellner SP, Colwell R, Bouma MJ. Cholera Dynamics and El Nino-Southern Oscillation. Science. 2000; 289 (5485): 1766-9.
49. Institute of One World Health. Diarrheal disease Fact Sheet. 2000.
Available from URL: http://www.oneworldhealth.org
50. Parashar UD, Bresee JS, Glass RI. The global burden of diarrhoeal disease in children. Bull World Health Organ 2003; 81 (4): 236.
51. Environmental Health Project. Diarrhea Prevention. Lessons Learned. 1999.
Available from URL: http://www.ehproject.org/PDF/Lessons_Learned
52. Patwari AK. Diarrhoea and malnutrition interaction. Indian J Paediatr. 1999; 66 (1 Suppl): S124-34.
53. Checkley W, Epstein LD, Gilman RH, Cabrera L, Black RE. Effects of acute diarrhea on linear growth in Peruvian children. Am J Epidemiol. 2003; 157 (2):166-75.
54. Moffat T. Diarrhea, respiratory infections, protozoan gastrointestinal parasites, and child growth in Kathmandu, Nepal. Am J Phys Anthropol. 2003;122(1):85-97.
55. Briend A, Hasan KZ, Aziz KM, Hoque BA. Are diarrhea control programmes likely to reduce childhood malnutrition. Observations from rural Bangladesh. Lancet. 1989; 2 (8658): 319-22.
56. Victora CG, Bryce J, Fontaine O, Monasch R. Reducing deaths from diarrhoea through oral rehydration therapy. Bull World Health Organ. 2000; 78 (10): 1246-55.
57. The Environmental Health Project. Preventing child diarrheal disease. 1999.
Available from URL: http://www.ehproject.org
58. The State of the World's Children, UNICEF, 2001.
Available from URL: http://www.unicef.org/sowc01
59. World Health Organization. Persistent diarrhea in children in developing countries: memorandum from a WHO meeting. Bull World Health Organ.1988; 66 (6): 709-11.
60. World Health Organization. Health and Environment in Sustainable Development. Five Years after the Earth Summit. 1997
Available from URL: http://www.who.int/docstore/peh/archives/execsum.htm

61. Reducing Deaths Due to Diarrhea.
Available from URL: http://www.basics.org/pdf/worldsummit
62. Geographycards. Vietnam geography. Available from URL:
http://www.geographycards.com/countryinfo/vm.html#Ge
63. Lonely planet. World Guide. Vietnam environment. Available from URL:
http://www.lonelyplanet.com/destinations/south_east_asia/vietnam/environment.htm
64. Sadec Asia Pacific. Vietnam diary. Fact Sheet. Geography and climate. 1999-2000.
Available from URL: http://www.sadec.com/Profile/viet05.html
65. The climate of Vietnam.
Available from URL: http://www.vietnamembassy.org.uk/climate.html
66. Vietnam at a glance.
Available from URL: http://www.worldbank.org/data/countrydata/aag/vnm_aag.pdf
67. Adams SJ. Vietnam's Health Care System: A Macroeconomic Perspective. The International Symposium on Health Care Systems in Asia, Hitotsubashi University, Tokyo, 2005.
Available from URL: http://www.imf.org/external/country/VNM/rr/sp/012105.pdf
68. National Assembly lays out health care reform. Vietnam News. 2005 Mar 12.
Available from URL:
http://vietnamnews.vnagency.com.vn/showarticle.php?num=03HEA120305
69. Nguyen VM et al. The Epidemiology and Disease Burden of Rotavirus in Vietnam: Sentinel Surveillance at 6 Hospitals. J Infect Dis. 2001; 183 (12):1707–12.
70. National Institute of Hygiene and Epidemiology. Statistical data on 24 communicable diseases in Vietnam. Unpublished data, 1990-2003.
71. Isenbarger DW, Hien BT, Ha HT, Ha TT, Bodhidatta L, Pang LW, Cam PD. Prospective study of the incidence of diarrhoea and prevalence of bacterial pathogens in a cohort of Vietnamese children along the Red River. Epidemiol Infect. 2001; 127(2): 229-36.
72. Thiem VD, Sethabutr O et al. Detection of Shigellae by a PCR Assay Targeting the ipaH Gene Suggests Increased Prevalence of Shigellosis in Nha Trang, Vietnam. J Clin Microbiol. 2004; 42 (5): 2031-35.

73. Nguyen TV, Van PL, Chinh LH, Khanh NG, Weintraub A. Detection and Characterization of Diarrheagenic Escherichia coli from Young Children in Hanoi, Vietnam. J Clin Microbiol. 2005; 43 (2): 755–60.
74. Hennekens CH, Buring JE. Epidemiology in Medicine. Wolters Kluwer Company, USA, 1987, p.132-49.
75. Altman DG. Practical statistics for medical research. Chapman and Hall, Great Britain, 1991, p.93-99.
76. Timmreck TC. An introduction to epidemiology. 3rd ed. Jones and Bartlett publishers, 2002, p.55-91.
77. Centre for Evidence-Based Medicine, Institute of Health Sciences. Study design. University of Oxford.
Available from URL: http://www.cebm.net/study_designs.asp
78. Katz MH. Multivarible analysis – A practical guide for clinicians. Cambridge University Press. 2nd ed. 2006, p.14-23.
79. Hennekens CH, Buring JE. Epidemiology in Medicine. Wolters Kluwer Company, USA, 1987, p.287-304.
80. Nguyen TV, Van PL, Chinh LH, Weintraub A. Diarrhea caused by rotavirus in children less than 5 years of age in Hanoi, Vietnam. J Clin Microbiol. 2004; 42(12):5745-50.
81. Ono K et al. Seasonal distribution of enteropathogens detected from diarrheal stool and water samples collected in Kathmandu, Nepal. Southeast Asian J Trop Med Public Health. 2001; 32(3):520-6.
82. Fewtrell L, Kaufmann RB, Kay D, Enanoria W, Haller L, Colford JM Jr. Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. Lancet Infect Dis. 2005; 5 (1): 42-52.
83. Fewtrell L, Colford JM Jr. Water, sanitation and hygiene in developing countries: interventions and diarrhoea--a review. Water Sci Technol. 2005; 52 (8): 133-42.

ANNEX 2

CONSENT FORM

I am ………………………….. from a research team established by Department of International Health, University of Oslo-Norway, Department of Enteric Bacteria-National Institute of Hygiene and Epidemiology, and Dong Anh Hospital. I am here to conduct a study on diarrhea morbidity among children less than five years of age admitted to district hospital. The study is trying to find out the most common causative agents and to understand the important factors associated with diarrhea morbidity among children less than five so that we could employ proper measures toward its prevention. Since the child is too young to decide on his/her own, I would like to interview you, and ask you for your permission to collect stool sample from your child.

I have few questions about diarrhea and related issues. Your answers will be written and then used for analysis. All information you provide will be handled as confidential and your individual answers will not be known, excepting the interviewer and the coordinator of this study. The results will be used only to improve strategies for prevention of dirrhea, one of the most common diseases among children in the community.

We will need at least 30 minutes to discuss and record the information. You can withdraw from the interview at any stage without any consequence if you do not wish to continue.

Will you participate in this study? Yes ☐ No ☐

Do you have any question?

Thank you.

Date: …../……./2005.

Interviewee's signature: …………………

Interviewer's signature: …………………

ANNEX 2

QUESTIONNAIRE

I. DEMOGRAPHIC AND SOCIO-ECONOMIC INFORMATION *Case/Control*: ………

1. Identification number: …………………………..

2. Address: ………………………………………………………………………………

3. Your age: ……years. The child's age: …………

4. The child's sex (Put √ in the applicable box) ☐ Male ☐ Female

5. Education: ☐ 1. Illiteracy
   2. Primary
   3. Junior secondary
   4. Senior secondary
   5. Bachelor.

6. Marital status: ☐ 1. Single 4. Widowed
   2. Married 5. Separated
   3. Divorced

7. Ethnic: ☐ 1. Kinh 3. Nung
   2. Tay 4. Other:

8. Occupation: Mother ☐ 1. Peasant
   Father ☐ 2. Employed
   3. Self-employed

9. Your family's income per month: ☐ 1. < 500 NOK
   2. 500-1000 NOK
   3. > 1000 NOK

10. How many surviving siblings does your child have? ☐ Older
   ☐ Younger

12. How many rooms are there in your house? ☐

13. How many people are living in this house? ……….

14. Do you have following items in your house? Television☐ Fridge☐ CD player☐
   Motobike☐ Radio ☐ Bicycle ☐

15. Do you have following kinds of livestock? If yes, state size of herd.

Cattle ☐	……….	Pig ☐	……………
Goats ☐	……….	Chickens ☐	……………
Dog ☐	………..	Other: ……………………	

II. CLINICAL DATA *(for case only)*

1. Hospitalized on: ……/……. / 2005

2. Weight: ……kg. Height: ……cm.

3. Temperature: …….. ^{0} C.

4. Number of days with diarrhea: ..…… days.

5. Stool frequency per day: ……

6. Is there blood in stool? Yes ☐ No ☐

7. Has the child vomited? Yes ☐ No ☐

   If yes, state vomiting frequency per day: ☐

8. Patient's dehydration status: ☐ 1. None
   2. Mild
   3. Moderate
   4. Severe

9. Did the child contact to any diarrheal patient for the last 7 days? Yes ☐ No ☐

10. Did the child eat any food sold by street vendors for the last 7 days? Yes ☐ No ☐

If yes, state what food the child ate ………………………………………………………..

11. Treatment before hospitalisation: ………………………………………………………..

…………………………………………………………………………………………..

…………………………………………………………………………………………..

12. Treatment during hospitalisation:

…………………………………………………………………………………………..

…………………………………………………………………………………………..

……………………………………………………………………………………..……

………………..……………………………………………………………………………

12. Stool sample collected on: …../……/2005.

III. Knowledge of diarrhea

1. Do you know diarrhea? Yes ☐ No ☐

   If no, go to Part IV.

2. If yes, what are the main signs/symptoms of diarrhea?

2.1. Three or more unformed stools within a day	☐	2.5. Vomiting	☐
2.2. Abdominal pain	☐	2.6. Fever	☐
2.3. Cramps	☐	2.7. Blood in stool	☐
2.4. Nausea	☐	2.6. Other: ………………	

3. What do you think causes diarrhea in young children?

3.1. Indigestible foods	☐	3.3. Teething	☐
3.2. Worm infection	☐	3.4. Other: ………………………………	
3.3. Germ infection	☐	3.5. Don't know	☐

4. What do you think spreads diarrhea?

   - …………………………………….   - ………………………………
   - …………………………………..   - ………………………………
   - …………………………………….   - ………………………………

5. Do you think diarrhea is a hazard to the child's health? Yes ☐ No ☐

6. Do you know some of the ways for preventing diarrhea? Yes ☐ No ☐

   If yes, mention some of them:

   - ...…………………………………………………………………………….
   - ……………………………………………………………………………….
   - ……………………………………………………………………………….

7. How did you know about diarrhea, signs, mode of spread and prevention?

7.1. School	☐	7.5. Hospital	☐
7.2. Television	☐	7.6. Friends	☐
7.3. Reading	☐	7.7. Village health worker	☐
7.4. Radio	☐	7.8. Other: ………………………………	

IV. Sanitation and rubbish diposal

1. Do you have a latrine? Yes ☐ No ☐

   If no, how do you defecate yourself? ☐ 1. Directly excrete into fishpond

   2. Directly excrete on the ground

   3. Other: ……………………

   If yes, is it in use? Yes in use ☐ Not in use ☐

2. Is it private of public? Private ☐ Public ☐

3. Type of the latrine used ☐ 1. Modern toilet.
2. Two-compartment latrine
3. Dry latrine
4. Other: …………….

4. How often is the latrine cleaned? ☐ 1. Every time it is spoiled
2. Every day
3. 1-2 times a week
4. Not cleaned

5. How many people use the latrine? ☐☐

6. Do you think no cleaning of latrine can facilitate to spread diarrhea? Yes ☐ No ☐

7. Are your children able to use the latrine on their own? Yes ☐ No ☐
If no, where do they defecate? …………………………………………….

8. If no in question 7, ☐ 1. Buried
How do you dispose of the feces? 2. Put in the latrine
3. Thrown away in open surrounding
4. Other: ……………………………

9. What care is given to children after going to toilet? ☐ 1. With water 2. With paper
3. Other: ………..
4. Not clean at all

10. Where do you dispose of household garbage? ☐ 1. Rubbish pit
2. Open surrounding
3. Other: ………………..

11. Where do you dispose of waste water? ☐ 1. Sewage system
2. Pond; 3. Garden
4. Other: …..…

12. Do you use stool as fertilizer Yes ☐ No ☐

V. HYGIENE PRACTICES AND OTHER DOMESTIC BEHAVIORS

1. Does your child feed on his/her own ? Yes ☐ No ☐

2. If yes, how does the child feed on his/her own? ☐ 1. With spoon
If no, go to question 5
2. With his/her hands
3. Other:………….

3. Do you often wash the child's hands before eating? ☐ Yes ☐ No

4. If yes, how do you treat the child's hands before eating any food? ☐ 1. Washing by water only
2. Washing by water with soap
3. Othes:………………

5. Do you often wash your hands?

5.1. After going to toilet ☐ 1. Never

5.2. Ater helping your child defecate? ☐ 2. Sometimes

5.3. Before eating and feeding your child ☐ 3. Usually

5.4. Before preparing foods for your child ☐

6. How do you wash your hands? ☐ 1. Water only; 2. Water and soap 3. Other:………………

7. Do you think that not washing your hands at 4 critical times in Question 5 as well as No hand-washing for the child before eating can spread diarrhea? Yes ☐ No ☐

8. Do you store cooked foods for later used? Yes ☐ No ☐

9. If yes, how do you store the cooked foods? ☐ 1. In refrigerator 2. In larder 3. In disk-cover 4. Other:…………….

10. How long do you often keep the cooked food before reuse? …………………….

11. Do you often heat the cooked foods before reuse? Yes ☐ No ☐

12. What do you use to clean utensils/containers for feeding your child ? ☐ 1. Water only 2. Hot water only 3. Water with soap 4. Hot water with soap

13. Do you often buy foods from street vendors for your child? Yes ☐ No ☐

14. How often do you clean your kitchen?

…………………………………………………...

15. Do flies present in the kitchen? Yes ☐ No ☐

16. Do animals enter the kitchen? Yes ☐ No ☐

17. Do you keep animals in the home overnight? Yes ☐ No ☐

VI. WATER RELATED PRACTICES

1. From what sources do you get your drinking water? ☐ 1. Running water 2. River 3. Pond 4. Well 5. Rain-water 6. Other:…….

2. What treatment is given to water before carrying home? ☐ 1. Filtering 2. Chlorinating 3. Using alum 4. Other:……. 5. None

3. What kind of utensils do you use for storing water? ☐ 1. Storage containers without lid
2. Storage containers with lid

4. Do you always clean/empty the storage container before replacing with fresh water? ☐ Yes ☐ No

5. What type of water does your family use for drinking? ☐ 1. Boiled 2. Filtered 3. Other: ……. 4. Untreated

6. Where do you often bath your child? ☐ 1. River 2. Pond 3. Bathroom 4. Other………

VII. BREASTFEEDING AND VACCINATION STATUSES

1. Do you breastfeed your child? Yes ☐ No ☐

2. If yes, have you exclusively breastfed the child in the first six month of his/her life?
Yes ☐ No ☐

3. If the child less than 6 months old, Have you exclusively been breastfeeding the child to date? Yes ☐ No ☐

If no, how long by now have you introduced other foods to the child? ……………

4. Do you know that breastfeeding adequately will reduce infections in a child? Yes ☐
No ☐

5. Has the child been vaccinated against measles? Yes ☐ No ☐

6. What other vaccines has the child been vaccinated? …………………………………

…………………………………………………………………………………………..

…………………………………………………………………………………………..

Dong Anh , ……./……./2005

Interviewer’s signature:………………………………